(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 February 2003 (27.02.2003)

PCT

(10) International Publication Number
WO 03/016482 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number: PCT/US02/26047

(22) International Filing Date: 16 August 2002 (16.08.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/312,758 17 August 2001 (17.08.2001) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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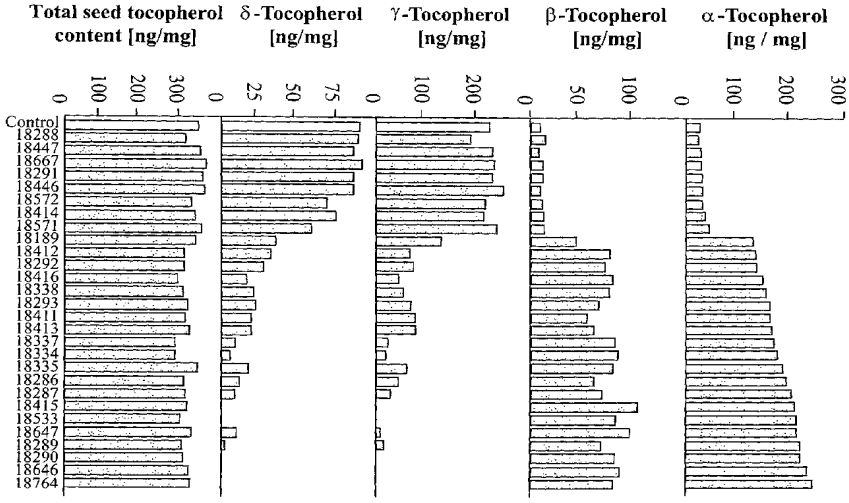
(54) Title: METHYLTRANSFERASE GENES AND USES THEREOF



(57) Abstract: The present invention relates to genes associated with the tocopherol biosynthesis pathway. More particularly, the present invention provides and includes nucleic acid molecules, proteins, and antibodies associated with genes that encode polypeptides that have methyltransferase activity. The present invention also provides methods for utilizing such agents, for example in gene isolation, gene analysis and the production of transgenic plants. Moreover, the present invention includes transgenic plants modified to express the aforementioned polypeptides. In addition, the present invention includes methods for the production of products from the tocopherol bisoynthesis pathway.

WO 03/016482 A2

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

METHYLTRANSFERASE GENES AND USES THEREOF

FIELD OF THE INVENTION

The present invention is in the field of plant genetics and biochemistry. More specifically, the invention relates to genes associated with the tocopherol biosynthesis pathway, namely those encoding methyltransferase activity, and uses of such genes.

BACKGROUND

Tocopherols are an important component of mammalian diets. Epidemiological evidence indicates that tocopherol supplementation can result in decreased risk for cardiovascular disease and cancer, can aid in immune function, and is associated with prevention or retardation of a number of degenerative disease processes in humans (Traber and Sies, *Annu. Rev. Nutr. 16*:321-347 (1996)). Tocopherol functions, in part, by stabilizing the lipid bilayer of biological membranes (Skrypin and Kagan, *Biochim. Biophys. Acta 815*:209 (1995); Kagan, *N.Y. Acad. Sci.* p 121, (1989); Gomez-Fernandez *et al.*, *Ann. N.Y. Acad. Sci.* p 109 (1989)), reducing polyunsaturated fatty acid (PUFA) free radicals generated by lipid oxidation (Fukuzawa *et al.*, *Lipids 17*: 511-513 (1982)), and scavenging oxygen free radicals, lipid peroxy radicals and singlet oxygen species (Diplock *et al. Ann. N Y Acad. Sci.* 570: 72 (1989); Fryer, *Plant Cell Environ. 15(4)*:381-392 (1992)).

α-Tocopherol, often referred to as vitamin E, belongs to a class of lipid-soluble antioxidants that includes α, β, γ, and δ-tocopherols and α, β, γ, and δ-tocotrienols. Although α, β, γ, and δ-tocopherols and α, β, γ, and δ-tocotrienols are sometimes referred to collectively as "vitamin E", vitamin E is more appropriately defined chemically as α-tocopherol. α-Tocopherol is significant for human health, in part because it is readily absorbed and retained by the body, and therefore has a higher degree of bioactivity than other tocopherol species (Traber and Sies, *Annu. Rev. Nutr. 16*:321-347 (1996)). However, other tocopherols such as β, γ, and δ-tocopherols, also have significant health and nutritional benefits.

Tocopherols are primarily synthesized only by plants and certain other photosynthetic organisms, including cyanobacteria. As a result, mammalian dietary tocopherols

are obtained almost exclusively from these sources. Plant tissues vary considerably in total tocopherol content and tocopherol composition, with α-tocopherol the predominant tocopherol species found in green, photosynthetic plant tissues. Leaf tissue can contain from 10-50 μg of total tocopherols per gram fresh weight, but most of the world's major staple crops (*e.g.*, rice, corn, wheat, potato) produce low to extremely low levels of total tocopherols, of which only a small percentage is α-tocopherol (Hess, Vitamin E, α-tocopherol, *In* Antioxidants in Higher Plants, R. Alscher and J. Hess, Eds., CRC Press, Boca Raton. pp. 111-134 (1993)). Oil seed crops generally contain much higher levels of total tocopherols, but α-tocopherol is present only as a minor component in most oilseeds (Taylor and Barnes, *Chem. Ind., Oct.*:722-726 (1981)).

The recommended daily dietary intake of 15-30 mg of vitamin E is quite difficult to achieve from the average American diet. For example, it would take over 750 grams of spinach leaves in which α-tocopherol comprises 60% of total tocopherols, or 200-400 grams of soybean oil to satisfy this recommended daily vitamin E intake. While it is possible to augment the diet with supplements, most of these supplements contain primarily synthetic vitamin E, having eight stereoisomers, whereas natural vitamin E is predominantly composed of only a single isomer. Furthermore, supplements tend to be relatively expensive, and the general population is disinclined to take vitamin supplements on a regular basis. Therefore, there is a need in the art for compositions and methods that either increase the total tocopherol production or increase the relative percentage of α-tocopherol produced by plants.

In addition to the health benefits of tocopherols, increased α-tocopherol levels in crops have been associated with enhanced stability and extended shelf life of plant products (Peterson, *Cereal-Chem. 72(1)*:21-24 (1995); Ball, *Fat-soluble vitamin assays in food analysis. A comprehensive review,* London, Elsevier Science Publishers Ltd. (1988)). Further, tocopherol supplementation of swine, beef, and poultry feeds has been shown to significantly increase meat quality and extend the shelf life of post-processed meat products by retarding post-processing lipid oxidation, which contributes to the undesirable flavor components (Sante and Lacourt, *J. Sci. Food Agric. 65(4)*:503-507 (1994); Buckley *et al.*, *J. of Animal Science 73*:3122-3130 (1995)).

TOCOPHEROL BIOSYNTHESIS

The plastids of higher plants exhibit interconnected biochemical pathways leading to secondary metabolites including tocopherols. The tocopherol biosynthetic path-

way in higher plants involves condensation of homogentisic acid and phytylpyrophosphate to form 2-methyl-6 phytylplastoquinol (Fiedler *et al.*, *Planta 155*: 511-515 (1982); Soll *et al.*, *Arch. Biochem. Biophys. 204*: 544-550 (1980); Marshall *et al.*, *Phytochem. 24*: 1705-1711 (1985)). This plant tocopherol pathway can be divided into four parts: 1) synthesis of homogentisic acid, which contributes to the aromatic ring of tocopherol; 2) synthesis of phytylpyrophosphate, which contributes to the side chain of tocopherol; 3) joining of HGA and phytylpyrophosphate via a prenyltransferase followed by a subsequent cyclization; 4) and S-adenosyl methionine dependent methylation of an aromatic ring, which affects the relative abundance of each of the tocopherol species.

SYNTHESIS OF HOMOGENTISIC ACID

Homogentisic acid is the common precursor to both tocopherols and plastoquinones. In at least some bacteria the synthesis of homogentisic acid is reported to occur via the conversion of chorismate to prephenate and then to p-hydroxyphenylpyruvate via a bifunctional prephenate dehydrogenase. Examples of bifunctional bacterial prephenate dehydrogenase enzymes include the proteins encoded by the *tyrA* genes of *Erwinia herbicola* and *Escherichia coli*. The *tyrA* gene product catalyzes the production of prephenate from chorismate, as well as the subsequent dehydrogenation of prephenate to form p-hydroxyphenylpyruvate (p-HPP), the immediate precursor to homogentisic acid. p-HPP is then converted to homogentisic acid by hydroxyphenylpyruvate dioxygenase (HPPD). In contrast, plants are believed to lack prephenate dehydrogenase activity, and it is generally believed that the synthesis of homogentisic acid from chorismate occurs via the synthesis and conversion of the intermediate arogenate. Since pathways involved in homogentisic acid synthesis are also responsible for tyrosine formation, any alterations in these pathways can also result in the alteration in tyrosine synthesis and the synthesis of other aromatic amino acids.

SYNTHESIS OF PHYTYLPYROPHOSPHATE

Tocopherols are a member of the class of compounds referred to as the isoprenoids. Other isoprenoids include carotenoids, gibberellins, terpenes, chlorophyll and abscisic acid. A central intermediate in the production of isoprenoids is isopentenyl diphosphate (IPP). Cytoplasmic and plastid-based pathways to generate IPP have been reported. The cytoplasmic based pathway involves the enzymes acetoacetyl CoA thio-

lase, HMGCoA synthase, HMGCoA reductase, mevalonate kinase, phosphomevalonate kinase, and mevalonate pyrophosphate decarboxylase.

Recently, evidence for the existence of an alternative, plastid based, isoprenoid biosynthetic pathway emerged from studies in the research groups of Rohmer and Arigoni (Eisenreich *et al.*, *Chem. Bio.*, *5*:R221-R233 (1998); Rohmer, *Prog. Drug. Res.*, *50*:135-154 (1998); Rohmer, *Comprehensive Natural Products Chemistry*, Vol. 2, pp. 45-68, Barton and Nakanishi (eds.), Pergamon Press, Oxford, England (1999)), who found that the isotope labeling patterns observed in studies on certain eubacterial and plant terpenoids could not be explained in terms of the mevalonate pathway. Arigoni and coworkers subsequently showed that 1-deoxyxylulose, or a derivative thereof, serves as an intermediate of the novel pathway, now referred to as the MEP pathway (Rohmer *et al.*, *Biochem. J.*, *295*:517-524 (1993); Schwarz, Ph.D. thesis, Eidgenössiche Technische Hochschule, Zurich, Switzerland (1994)). Recent studies showed the formation of 1-deoxyxylulose 5-phosphate (Broers, Ph.D. thesis (Eidgenössiche Technische Hochschule, Zurich, Switzerland) (1994)) from one molecule each of glyceraldehyde 3-phosphate (Rohmer, *Comprehensive Natural Products Chemistry*, Vol. 2, pp. 45-68, Barton and Nakanishi, eds., Pergamon Press, Oxford, England (1999)) and pyruvate (Eisenreich *et al.*, *Chem. Biol.*, *5*:R223-R233 (1998); Schwarz *supra*; Rohmer *et al.*, *J. Am. Chem. Soc.*, *118*:2564-2566 (1996); and Sprenger *et al.*, *Proc. Natl. Acad. Sci. USA*, *94*:12857-12862 (1997)) by an enzyme encoded by the *dxs* gene (Lois *et al.*, *Proc. Natl. Acad. Sci. USA*, *95*:2105-2110 (1997); and Lange *et al.*, *Proc. Natl. Acad. Sci. USA*, *95*:2100-2104 (1998)). 1-Deoxyxylulose 5-phosphate can be further converted into 2-*C*-methylerythritol 4-phosphate (Arigoni *et al.*, *Proc. Natl. Acad. Sci. USA*, *94*:10600-10605 (1997)) by a reductoisomerase encoded by the *dxr* gene (Bouvier *et al.*, *Plant Physiol*, *117*:1421-1431 (1998); and Rohdich *et al.*, *Proc. Natl. Acad. Sci. USA*, *96*:11758-11763 (1999)).

Reported genes in the MEP pathway also include *ygbP*, which catalyzes the conversion of 2-C-methylerythritol 4-phosphate into its respective cytidyl pyrophosphate derivative and *ygbB*, which catalyzes the conversion of 4-phosphocytidyl-2C-methyl-D-erythritol into 2C-methyl-D-erythritol, 3, 4-cyclophosphate. These genes are tightly linked on the *E. coli* genome (Herz *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, *97(6)*:2485-2490 (2000)).

Once IPP is formed by the MEP pathway, it is converted to GGDP by GGDP synthase, and then to phytylpyrophosphate, which is the central constituent of the tocopherol side chain.

COMBINATION AND CYCLIZATION

Homogentisic acid is combined with either phytyl-pyrophosphate or solanyl-pyrophosphate by phytyl/prenyl transferase forming 2-methyl-6-phytyl plastoquinol or 2-methyl-6-solanyl plastoquinol, respectively. 2-methyl-6-solanyl plastoquinol is a precursor to the biosynthesis of plastoquinones, while 2-methyl-6-phytyl plastoquinol is ultimately converted to tocopherol.

METHYLATION OF THE AROMATIC RING

The major structural difference between each of the tocopherol subtypes is the position of the methyl groups around the phenyl ring. Both 2-methyl-6-phytyl plastoquinol and 2-methyl-6-solanyl plastoquinol serve as substrates for 2-methyl-6-phytyl-plastoquinol/2-methyl-6-solanylplastoquinol-9 methyltransferase (Methyl Transferase 1; MT1), which catalyzes the formation of plastoquinol-9 and γ-tocopherol respectively, by methylation of the 7 position. Subsequent methylation at the 5 position of γ-tocopherol by γ-tocopherol methyl-transferase (GMT) generates the biologically active α-tocopherol. Additional potential MT1 substrates include 2-methyl-5-phytylplastoquinol and 2-methyl-3-phytylplastoquinol. Additional potential substrates for GMT include δ-tocopherol and γ- and δ-tocotrienol.

There is a need in the art for nucleic acid molecules encoding enzymes involved in tocopherol biosynthesis, as well as related enzymes and antibodies for the enhancement or alteration of tocopherol production in plants. There is a further need for transgenic organisms expressing those nucleic acid molecules involved in tocopherol biosynthesis, which are capable of nutritionally enhancing food and feed sources.

SUMMARY OF THE INVENTION

The present invention includes and provides a substantially purified nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2-17, 50, and 85.

The present invention includes and provides a substantially purified nucleic acid molecule comprising a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO: 19-31 and 33-38.

The present invention includes and provides a substantially purified nucleic acid molecule comprising as operably linked components: (A) a promoter region which functions in a plant cell to cause the production of an mRNA molecule; (B) a heterologous nucleic acid molecule encoding a polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 19-31, 33-41.

The present invention includes and provides a substantially purified protein comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 19-31, and 33-38.

The present invention includes and provides an antibody capable of specifically binding a substantially purified protein comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 19-31, and 33-38.

The present invention includes and provides a transformed plant having an exogenous nucleic acid molecule that encodes a polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 19-31, and 33-41.

The present invention includes and provides a transformed plant having an exogenous nucleic acid molecule that encodes a polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 46-49.

The present invention includes and provides a method for reducing expression of MT1 or GMT in a plant comprising: (A) transforming a plant with a nucleic acid molecule, said nucleic acid molecule having an exogenous promoter region which functions in plant cells to cause the production of a mRNA molecule, wherein said exogenous promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand, wherein said transcribed strand is complementary to a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2-17, 50, and 85; and wherein said transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the plant cells to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA sequence; and (B) growing said transformed plant.

The present invention includes and provides a transformed plant comprising a nucleic acid molecule comprising an exogenous promoter region which functions in plant cells to cause the production of a mRNA molecule, wherein the exogenous promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand, wherein the transcribed strand is complementary to a

nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2-17, 50, 85, and wherein the transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the plant cells to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA sequence; wherein, the expression of MT1, GMT or both is reduced relative to a plant with a similar genetic background but lacking the exogenous nucleic acid molecule.

The present invention includes and provides method for increasing the γ-tocopherol content in a plant comprising: (A) transforming a plant with a nucleic acid molecule, the nucleic acid molecule comprising an exogenous promoter region which functions in plant cells to cause the production of a mRNA molecule, wherein the exogenous promoter region is linked to a transcribed nucleic acid molecule comprising a transcribed strand and a non-transcribed strand, wherein the transcribed strand is complementary to a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2-17, 50, and 85; and wherein the transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the plant cells to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA sequence; and (C) growing the transformed plant.

The current invention further includes and provides a transformed plant comprising: (A) a first nucleic acid molecule comprising an exogenous promoter region which functions in plant cells to cause the production of a mRNA molecule, wherein the exogenous promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand, wherein the transcribed strand is complementary to a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2-17, 50, and 85, and wherein the transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the plant cells to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA sequence; and (B) a second nucleic acid molecule comprising an exogenous promoter region which functions in plant cells to cause the production of a mRNA molecule, wherein the exogenous promoter region is linked to a nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 42-45, wherein the γ-tocopherol content of the transformed plant is increased relative to a plant with a similar genetic background but lacking the exogenous nucleic acid molecule.

The present invention includes and provides a method of producing a plant having a seed with an increased α-tocopherol level comprising: (A) transforming the plant with a nucleic acid molecule, wherein the nucleic acid molecule comprises a sequence encoding a polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 19-31, 33-38, and 39-41; and (B) growing the transformed plant.

The present invention includes and provides a method of producing a plant having a seed with an increased γ-tocopherol level comprising: (A) transforming the plant with a nucleic acid molecule, wherein the nucleic acid molecule comprises a nucleic acid sequence that encodes a polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 46-49; and (B) growing the transformed plant.

The present invention includes and provides a method of accumulating α-tocopherol in a seed comprising: (A) growing a plant with a heterologous nucleic acid molecule, wherein the heterologous nucleic acid molecule comprises a sequence encoding a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 19-31, 33-38, and 39-41; and (B) isolating said seed from said plant with a heterologous nucleic acid molecule.

The present invention includes and provides a method of accumulating γ-tocopherol in a seed comprising: (A) growing a plant with a heterologous nucleic acid molecule, wherein the heterologous nucleic acid molecule comprises a sequence encoding a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 46-49; and (B) isolating said seed from said plant with a heterologous nucleic acid molecule.

The present invention includes and provides a seed derived from a transformed plant having an exogenous nucleic acid molecule comprising a nucleic acid sequence encoding an polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 19-31, 33-38, and 39-41, wherein the seed has an increased α-tocopherol level relative to seeds from a plant having a similar genetic background but lacking the exogenous nucleic acid molecule.

The present invention includes and provides an oil derived from a seed of a transformed plant, wherein the transformed plant contains an exogenous nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide molecule

comprising a sequence selected from the group consisting of SEQ ID NOs: 19-31, 33-38, and 39-41.

The present invention includes and provides feedstock comprising a transformed plant or part thereof, wherein the transformed plant has an exogenous nucleic acid molecule that comprises a nucleic acid sequence encoding a polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NO s: 19-31, 33-38, and 39-41.

The present invention includes and provides a meal comprising plant material manufactured from a transformed plant, wherein the transformed plant has an exogenous nucleic acid molecule that comprises a nucleic acid sequence encoding a polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 19-31, 33-38, and 39-41.

The present invention includes and provides a seed derived from a transformed plant having an exogenous nucleic acid molecule comprising a sequence encoding a polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 46-49, wherein the seed has an increased tocopherol level relative to seeds from a plant having a similar genetic background but lacking the exogenous nucleic acid molecule.

The present invention includes and provides oil derived from a seed of a transformed plant, wherein the transformed plant contains an exogenous nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 46-49.

The present invention also includes and provides feedstock comprising a transformed plant or part thereof, wherein the transformed plant has an exogenous nucleic acid molecule that comprises a nucleic acid sequence encoding a polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 46-49.

The present invention also includes and provides meal comprising plant material manufactured from a transformed plant, wherein the transformed plant has an exogenous nucleic acid molecule that comprises a nucleic acid sequence encoding a polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NO: 46-49.

The present invention also includes and provides a host cell comprising a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2 -17, 42-45 and complements thereof.

The present invention also includes and provides an introduced first nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 19-31, 33-38, and 39-41, and an introduced second nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA, slr1736, ATPT2, dxs, dxr, GGPPS, HPPD, GMT, MT1, tMT2, AANT1, slr 1737*, and an antisense construct for homogentisic acid dioxygenase.

The present invention also includes and provides a transformed plant comprising an introduced first nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 46-49, and an introduced second nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA, slr1736, ATPT2, dxs, dxr, GGPPS, HPPD, GMT, MT1, tMT2, AANT1, slr 1737*, and an antisense construct for homogentisic acid dioxygenase.

The present invention also includes and provides a plant comprising an introduced nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 42-45, wherein said transformed plant produces a seed having increased total tocopherol relative to seed of a plant with a similar genetic background but lacking said introduced nucleic acid molecule.

The present invention also includes and provides a plant comprising an introduced nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2-17, 50, 85, wherein said transformed plant produces a seed having increased total tocopherol relative to seed of a plant with a similar genetic background but lacking said introduced nucleic acid molecule.

The present invention also includes and provides a plant comprising a first introduced nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2-17, 50, and 85 and a second introduced nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 42-45, wherein said transformed plant produces a seed having increased total tocopherol relative to seed of a plant with a similar genetic background but lacking both said introduced first nucleic acid molecule and said introduced second nucleic acid molecule.

DESCRIPTION OF THE NUCLEIC AND AMINO ACID SEQUENCES

SEQ ID NO: 1 sets forth a nucleic acid sequence of a DNA molecule that encodes an *Arabidopsis thaliana* gamma-tocopherol methyltransferase.

SEQ ID NO: 2 sets forth a nucleic acid sequence of a DNA molecule that encodes an *Arabidopsis thaliana,* Columbia ecotype, gamma-tocopherol methyltransferase.

SEQ ID NO: 3 sets forth a nucleic acid sequence of a DNA molecule that encodes an *Oryza sativa* gamma-tocopherol methyltransferase.

SEQ ID NO: 4 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Gossypium hirsutum* gamma-tocopherol methyltransferase.

SEQ ID NO: 5 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Cuphea pulcherrima* gamma-tocopherol methyltransferase.

SEQ ID NO: 6 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Brassica napus* S8 gamma-tocopherol methyltransferase.

SEQ ID NO: 7 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Brassica napus* P4 gamma-tocopherol methyltransferase.

SEQ ID NO: 8 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Brassica napus* S8 gamma-tocopherol methyltransferase.

SEQ ID NO: 9 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Brassica napus* P4 gamma-tocopherol methyltransferase.

SEQ ID NO: 10 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Lycopersicon esculentum* gamma-tocopherol methyltransferase.

SEQ ID NO: 11 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Glycine max* gamma-tocopherol methyltransferase 1.

SEQ ID NO: 12 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Glycine max* gamma-tocopherol methyltransferase 2.

SEQ ID NO: 13 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Glycine max* gamma-tocopherol methyltransferase 3.

SEQ ID NO: 14 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Tagetes erecta* gamma-tocopherol methyltransferase.

SEQ ID NO: 15 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Sorghum bicolor* gamma-tocopherol methyltransferase.

SEQ ID NO: 16 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Nostoc punctiforme* gamma-tocopherol methyltransferase.

SEQ ID NO: 17 sets forth a nucleic acid sequence of a DNA molecule that encodes an *Anabaena* gamma-tocopherol methyltransferase.

SEQ ID NO: 18 set forth a derived amino acid sequence of an *Arabidopsis thaliana* gamma-tocopherol methyltransferase.

SEQ ID NO: 19 sets forth a derived amino acid sequence of an *Arabidopsis thaliana,* Columbia ecotype, gamma-tocopherol methyltransferase.

SEQ ID NO: 20 sets forth a derived amino acid sequence of an *Oryza sativa* gamma-tocopherol methyltransferase.

SEQ ID NO: 21 sets forth a derived amino acid sequence of a *Zea mays* gamma-tocopherol methyltransferase.

SEQ ID NO: 22 sets forth a derived amino acid sequence of a *Gossypium hirsutum* gamma-tocopherol methyltransferase.

SEQ ID NO: 23 sets forth a derived amino acid sequence of a *Cuphea pulcherrima* gamma-tocopherol methyltransferase.

SEQ ID NO: 24 sets forth a derived amino acid sequence of a *Brassica napus* S8 gamma-tocopherol methyltransferase.

SEQ ID NO: 25 sets forth a derived amino acid sequence of a *Brassica napus* P4 gamma-tocopherol methyltransferase.

SEQ ID NO: 26 sets forth a derived amino acid sequence of a *Lycopersicon esculentum* gamma-tocopherol methyltransferase.

SEQ ID NO: 27 sets forth a derived amino acid sequence of a *Glycine max* gamma-tocopherol methyltransferase.

SEQ ID NO: 28 sets forth a derived amino acid sequence of a *Glycine max* gamma-tocopherol methyltransferase.

SEQ ID NO: 29 sets forth a derived amino acid sequence of a *Glycine max* gamma-tocopherol methyltransferase.

SEQ ID NO: 30 sets forth a derived amino acid sequence of a *Tagetes erecta* gamma-tocopherol methyltransferase.

SEQ ID NO: 31 sets forth a derived amino acid sequence of a *Sorghum bicolor* gamma-tocopherol methyltransferase.

SEQ ID NO: 32 sets forth an amino acid sequence of a pea rubisco small subunit chloroplast targeting sequence (CTP1).

SEQ ID NO: 33 sets forth a derived mature amino acid sequence of a *Brassica napus* S8 gamma-tocopherol methyltransferase.

SEQ ID NO: 34 sets forth a derived mature amino acid sequence of a *Brassica napus* P4 gamma-tocopherol methyltransferase.

SEQ ID NO: 35 sets forth a derived mature amino acid sequence of a *Cuphea pulcherrima* gamma-tocopherol methyltransferase.

SEQ ID NO: 36 sets forth a derived mature amino acid sequence of a *Gossypium hirsutum* gamma-tocopherol methyltransferase.

SEQ ID NO: 37 sets forth a derived mature amino acid sequence of a *Tagetes erecta* gamma-tocopherol methyltransferase.

SEQ ID NO: 38 sets forth a derived mature amino acid sequence of a *Zea mays* gamma-tocopherol methyltransferase.

SEQ ID NO: 39 sets forth a derived amino acid sequence of a *Nostoc punctiforme* gamma-tocopherol methyltransferase.

SEQ ID NO: 40 sets forth a derived amino acid sequence of an *Anabaena* gamma-tocopherol methyltransferase.

SEQ ID NO: 41 sets forth an amino acid sequence of *Synechocystis* gamma-tocopherol methyltransferase.

SEQ ID NO: 42 sets forth a nucleic acid sequence of a nucleic acid molecule encoding a *Synechocystis* pcc 6803 2-methyl-6-phytylplastoquinol/2-methyl-6-solanylplastoquinol-9 methyltransferase.

SEQ ID NO: 43 sets forth a nucleic acid sequence of a nucleic acid molecule encoding an *Anabaena* 2-methyl-6-phytylplastoquinol/2-methyl-6-solanylplastoquinol-9 methyltransferase.

SEQ ID NO: 44 sets forth a nucleic acid sequence of a nucleic acid molecule encoding a *Synechococcus* 2-methyl-6-phytylplastoquinol/2-methyl-6-solanylplastoquinol-9 methyltransferase.

SEQ ID NO: 45 sets forth a nucleic acid sequence of a nucleic acid molecule encoding a *Prochlorococcus marinus* 2-methyl-6-phytylplastoquinol/2-methyl-6-solanylplastoquinol-9 methyltransferase.

SEQ ID NO: 46 sets forth a derived amino acid sequence of an *Synechocystis* pcc 6803 2-methyl-6-phytylplastoquinol/2-methyl-6-solanylplastoquinol-9 methyltransferase.

SEQ ID NO: 47 sets forth a derived amino acid sequence of an *Anabaena* 2-methyl-6-phytylplastoquinol/2-methyl-6-solanylplastoquinol-9 methyltransferase.

SEQ ID NO: 48 sets forth a derived amino acid sequence of a *Synechococcus* 2-methyl-6-phytylplastoquinol/2-methyl-6-solanylplastoquinol-9 methyltransferase.

SEQ ID NO: 49 sets forth a derived amino acid sequence of a *Prochlorococcus* 2-methyl-6-phytylplastoquinol/2-methyl-6-solanylplastoquinol-9 methyltransferase.

SEQ ID NO: 50 sets forth a nucleic acid sequence of an *Oryza sativa* gamma-tocopherol methyltransferase.

SEQ ID NOs: 51 and 52 set forth a nucleic acid sequence of primers for use in amplifying a *Brassica napus* S8 gamma methyl transferase.

SEQ ID NOs: 53 and 54 set forth a nucleic acid sequence of primers for use in amplifying a *Brassica napus* P4 gamma methyl transferase.

SEQ ID NOs: 55 and 56 set forth a nucleic acid sequence of primers for use in amplifying a *Cuphea pulcherrima* gamma methyl transferase.

SEQ ID NOs: 57 and 58 set forth a nucleic acid sequence of primers for use in amplifying a *Gossypium hirsutum* gamma methyl transferase.

SEQ ID NOs: 59 and 60 set forth a nucleic acid sequence of primers for use in amplifying a mature *Brassica napus* S8 gamma methyl transferase and a mature *Brassica napus* P4 gamma methyl transferase.

SEQ ID NOs: 61 and 62 set forth a nucleic acid sequence of primers for use in amplifying a mature *Cuphea pulcherrima* gamma methyl transferase.

SEQ ID NOs: 63 and 64 set forth a nucleic acid sequence of primers for use in amplifying a mature *Gossypium hirsutum* gamma methyl transferase.

SEQ ID NOs: 65 and 66 set forth a nucleic acid sequence of primers for use in amplifying a mature *Tagetes erecta* gamma methyl transferase.

SEQ ID NOs: 67 and 68 set forth a nucleic acid sequence of primers for use in amplifying a *Nostoc punctiforme* gamma methyl transferase.

SEQ ID NOs: 69 and 70 set forth a nucleic acid sequence of primers for use in amplifying an *Anabaena* gamma methyl transferase.

SEQ ID NOs: 71 and 72 set forth a nucleic acid sequence of primers for use in amplifying an *Anabaena* 2-methyl-6-phytylplastoquinol/2-methyl-6-solanylplastoquinol-9 methyltransferase.

SEQ ID NOs: 73 and 74 set forth a nucleic acid sequence of primers for use in amplifying a mature *Zea mays* gamma methyl transferase.

SEQ ID NOs: 75 and 76 set forth a nucleic acid sequence of primers for use in amplifying an *Arabidopsis* gamma methyl transferase.

SEQ ID NOs: 77 and 78 set forth a nucleic acid sequence of primers for use in amplifying an *Arabidopsis* gamma methyl transferase.

SEQ ID NOs: 79 and 80 set forth a nucleic acid sequence of primers for use in amplifying an Arcelin 5 promoter.

SEQ ID NO: 81 sets forth a 5' translational start region of a nucleic acid sequence corresponding to an Arcelin 5 promoter from pARC5-1.

SEQ ID NO: 82 sets forth a 5' translational start region of a nucleic acid sequence corresponding to an Arcelin 5 promoter from pARC5-1M.

SEQ ID NOs: 83 and 84 set forth nucleic acid sequences of primers for use in amplifying an *Anabaena* putative-MT1 coding sequence.

SEQ ID NO: 85 sets forth a nucleic acid sequence of a *Zea mays* gamma-tocopherol methyltransferase.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 is a schematic of construct pET-DEST42.

FIGURE 2 is a schematic of construct pCGN9979.

FIGURE 3 is a schematic of construct pMON26592.

FIGURE 4 is a schematic of construct pMON26593.

FIGURE 5 is a schematic of construct pMON55524.

FIGURE 6 is a schematic of construct pMON36500.

FIGURE 7 is a schematic of construct pMON36501.

FIGURE 8 is a schematic of construct pMON36502.

FIGURE 9 is a schematic of construct pMON36503.

FIGURE 10 is a schematic of construct pMON36504.

FIGURE 11 is a schematic of construct pMON36505.

FIGURE 12 is a schematic of construct pMON36506.

FIGURE 13 is a schematic of construct pMON67157.

FIGURE 14 is a graph depicting the soy seed tocopherol content and composition from pooled seed of the R1 generation of plants transformed with pMON36503. This construct expresses an *A. thaliana* GMT under p7S promoter control.

FIGURE 15 is a graph depicting the soy seed tocopherol content and composition from pooled seed of the R1 generation of plants transformed with pMON36505. This construct expresses an *A. thaliana* GMT under arcelin5 promoter control.

FIGURE 16 is a graph depicting the soy seed tocopherol content and composition from pooled seed of the R1 generation of plants transformed with pMON36506. This construct expresses an *A. thaliana* GMT under the control of the modified arcelin 5 promoter.

FIGURE 17 is a graph representing the enzyme activities of various gamma-methyltransferases (GMT) and a tocopherol methyl transferase 1 (MT1) in recombinant *E. coli* crude extract preparations. Enzyme activities are expressed as either pmol α-tocopherol (GMT) or 2,3-dimethyl-5-phytylplastoquinol (MT1) formation per mg protein per min. Vector designations stand for the following recombinant genes: pMON67171, mature cotton GMT; pMON67173, mature *Cuphea pulcherrima* GMT; pMON67177, mature marigold GMT; pMON67181, mature *Brassica napus* S8 GMT; pMON67183, *Zea mays* GMT; pMON67175, *Anabaena* GMT; pMON67176, *Nostoc* GMT; and pMON67174, *Anabaena* MT1.

FIGURE 18 is an HPLC chromatogram, representing the methyltransferase activity of recombinant expressed *Anabaena* methyltransferase 1. Enzyme activity is monitored on crude cell extracts from *E. coli* harboring pMON67174.

FIGURE 19 is an HPLC chromatogram, representing the Methyltransferase activity of recombinant expressed *Anabaena* methyltransferase 1 without 2-methylphytylplastoquinol substrate (negative control). Enzyme activity is monitored on crude cell extracts from *E. coli* harboring pMON67174.

FIGURE 20 is an HPLC chromatogram, representing the methyltransferase 1 activity in isolated pea chloroplasts (positive control).

FIGURES 21A and 21B are graphs representing the α and γ-tocopherol levels in Arabidopsis T_2 seed from 5 transgenic control plants containing the napin binary vector (9979), 15 transgenic plants expressing the *Arabidopsis thaliana* GMT gene (Columbia ecotype) under the control of the napin promoter (67156) and 13 transgenic plants expressing the *Brassica napus* P4 GMT under the control of the napin promoter (67159).

FIGURES 22A and 22B are graphs representing the α and γ-tocopherol levels in *Arabidopsis* T_2 seeds from 5 transgenic plants containing the napin binary vector (9979), 15 transgenic plants expressing the *Cuphea pulcherrima* GMT gene under the control of

the napin promoter (67158) and 1 transgenic plant expressing the *Brassica napus* P4 GMT under the control of the napin promoter (67159).

FIGURE 23 is a graph representing the average seed γ-tocopherol level in transformed *Arabidopsis* plants harboring expression constructs for the *Arabidopsis thaliana* ecotype Columbia GMT (67156), the cuphea GMT (67158), the Brassica P4 GMT (67159), the cotton GMT (67160), and the Brassica S8 GMT (67170).

FIGURE 24 is a graph representing the average seed α-tocopherol level in transformed *Brassica* plants.

FIGURE 25 shows the percent of seed δ-tocopherol in *Arabidopsis* T2 seed from lines expressing MT1 under the control of the napin promoter.

FIGURE 26 shows T_3 seed δ-tocopherol levels in two lines expressing MT1 under the control of the napin promoter.

FIGURE 27 represents pMON67212.

FIGURE 28 represents pMON67213.

FIGURE 29 shows total tocopherol level for *Arabidopsis* transformed with an MT1 and prenyltransferase double construct.

FIGURE 30 shows γ tocopherol level for *Arabidopsis* transformed with an MT1 and prenyltransferase double construct.

FIGURE 31 shows δ-tocopherol level for *Arabidopsis* transformed with an MT1 and prenyltransferase double construct.

FIGURE 32 shows α-tocopherol level for *Arabidopsis* transformed with an MT1 and prenyltransferase double construct.

FIGURE 33 is a graph showing 2-Methylphytylplastoquinol methyltransferase activity obtained with recombinant proteins and a pea chloroplast control. Data are obtained with recombinant proteins from microbial and plant sources.

FIGURE 34 is a graph showing GMT substrate specificity for gamma-tocopherols versus gamma-tocotrienols. GMT activity is measured with recombinant expressed gamma methyltransferases from cotton, *Anabaena*, and corn, using gamma tocopherol or gamma-tocotrienol and S-adenosylmethionine as a substrate.

DETAILED DESCRIPTION

The present invention provides a number of agents, for example, nucleic acid molecules and polypeptides associated with the synthesis of tocopherol, and provides uses of such agents.

AGENTS

The agents of the invention will preferably be "biologically active" with respect to either a structural attribute, such as the capacity of a nucleic acid to hybridize to another nucleic acid molecule, or the ability of a protein to be bound by an antibody (or to compete with another molecule for such binding). Alternatively, such an attribute may be catalytic and thus involve the capacity of the agent to mediate a chemical reaction or response. The agents will preferably be "substantially purified." The term "substantially purified," as used herein, refers to a molecule separated from substantially all other molecules normally associated with it in its native state. More preferably a substantially purified molecule is the predominant species present in a preparation. A substantially purified molecule may be greater than 60% free, preferably 75% free, more preferably 90% free, and most preferably 95% free from the other molecules (exclusive of solvent) present in the natural mixture. The term "substantially purified" is not intended to encompass molecules present in their native state.

The agents of the invention may also be recombinant. As used herein, the term recombinant means any agent (*e.g.*, DNA, peptide *etc.*), that is, or results, however indirectly, from human manipulation of a nucleic acid molecule.

It is understood that the agents of the invention may be labeled with reagents that facilitate detection of the agent (*e.g.*, fluorescent labels, Prober *et al., Science 238*:336-340 (1987); Albarella *et al.*, EP 144914; chemical labels, Sheldon *et al.*, U.S. Patent 4,582,789; Albarella *et al.*, U.S. Patent 4,563,417; modified bases, Miyoshi *et al.*, EP 119448).

NUCLEIC ACID MOLECULES

Agents of the invention include nucleic acid molecules. In a preferred aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence, which encodes a gamma-tocopherol methyltransferase. As used herein a gamma-tocopherol methyltransferase (also referred to as GMT, γ-GMT, γ-MT, γ-TMT or gamma-methyltransferase) is any polypeptide that is capable of specifically catalyzing the conversion of γ-tocopherol into α-tocopherol. In certain plant species such as soybean, GMT can also catalyze the conversion of δ-tocopherol to β-tocopherol. In other plants, mainly monocotyledons such as corn and wheat, GMT can also catalyze the conversion of γ-tocotrienol to α-tocotrienol and δ-tocotrienol to β-tocotrienol. A preferred gamma-tocopherol methyltransferase is a plant or cynobacterial gamma-tocopherol methyltransfer-

ase, more preferably a gamma-tocopherol methyltransferase that is also found in an organism selected from the group consisting of *Arabidopsis,* rice, corn, cotton, cuphea, oilseed rape, tomato, soybean, marigold, sorghum, and leek, most preferably a gamma-tocopherol methyltransferase that is also found in an organism selected from the group consisting of *Arabidopsis thaliana, Oryza sativa, Zea mays, Gossypium hirsutum, Cuphea pulcherrima, Brassica napus, Lycopersicon esculentum, Glycine max, Tagetes erecta,* and *Lilium asiaticum.* An example of a more preferred gamma-tocopherol methyltransferase is a polypeptide with one of the amino acid sequences set forth in SEQ ID NOs: 19-31 and 33-38.

In another embodiment of the invention, genomic DNA is used to transform any of the plants disclosed herein. Genomic DNA (e.g. SEQ ID NOs: 6 and 7) can be particularly useful for transforming monocotyledonous plants (e.g. SEQ ID NOs: 6 and 7).

In another preferred aspect of the present invention the nucleic acid molecule of the invention comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2-17, 50, and 85, and complements thereof and fragments of either. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 19-31, 33, and 38 and fragments thereof.

In another preferred aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence, which encodes a 2-methyl-6-phytylplastoquinol/2-methyl-6-solanylplastoquinol-9 methyltransferase. As used herein a 2-methyl-6-phytylplastoquinol/2-methyl-6-solanylplastoquinol-9 methyltransferase (MT1) is any protein that is capable of specifically catalyzing the conversion of 2-methyl-6-phytylplastoquinol, 2-methyl-5-phytylplastoquinol or 2-methyl-3-phytylplastoquinol to 2,3-dimethyl-6-phytylplastoquinol. A preferred MT 1 is a cyanobacterial MT 1, more preferably an MT 1 that is also found in an organism selected from the group consisting of *Anabaena, Synechococcus* and *Prochlorococcus marinus.* An example of a more preferred MT 1 is a polypeptide with the amino acid sequence selected from the group consisting of SEQ ID NOs: 46-49.

In another preferred aspect of the present invention the nucleic acid molecule of the invention comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 42-45 and complements thereof and fragments of either. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence

encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 46-49 and fragments thereof.

In another preferred aspect of the present invention a nucleic acid molecule comprises nucleotide sequences encoding a plastid transit peptide operably fused to a nucleic acid molecule that encodes a protein or fragment of the present invention.

It is understood that in a further aspect of the present invention, the nucleic acids can encode a protein that differs from any of the proteins in that one or more amino acids have been deleted, substituted or added without altering the function. For example, it is understood that codons capable of coding for such conservative amino acid substitutions are known in the art.

One subset of the nucleic acid molecules of the invention is fragment nucleic acids molecules. Fragment nucleic acid molecules may consist of significant portion(s) of, or indeed most of, the nucleic acid molecules of the invention, such as those specifically disclosed. Alternatively, the fragments may comprise smaller oligonucleotides (having from about 15 to about 400 nucleotide residues and more preferably, about 15 to about 30 nucleotide residues, or about 50 to about 100 nucleotide residues, or about 100 to about 200 nucleotide residues, or about 200 to about 400 nucleotide residues, or about 275 to about 350 nucleotide residues).

A fragment of one or more of the nucleic acid molecules of the invention may be a probe and specifically a PCR probe. A PCR probe is a nucleic acid molecule capable of initiating a polymerase activity while in a double-stranded structure with another nucleic acid. Various methods for determining the structure of PCR probes and PCR techniques exist in the art. Computer generated searches using programs such as Primer3 (www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi), STSPipeline (www.genome.wi.mit.edu/cgi-bin/www-STS_Pipeline), or GeneUp (Pesole *et al.*, *BioTechniques 25*:112-123 (1998)), for example, can be used to identify potential PCR primers.

Another subset of the nucleic acid molecules of the invention include nucleic acid molecules that encode a polypeptide or fragment thereof.

Nucleic acid molecules or fragments thereof of the present invention are capable of specifically hybridizing to other nucleic acid molecules under certain circumstances. Nucleic acid molecules of the present invention include those that specifically hybridize to nucleic acid molecules having a nucleic acid sequence selected from the group con-

sisting of SEQ ID NOs: 2-17, 50, and 85, and complements thereof. Nucleic acid molecules of the present invention also include those that specifically hybridize to nucleic acid molecules encoding an amino acid sequence selected from SEQ ID NOs: 19-31 and 33-38 and fragments thereof.

As used herein, two nucleic acid molecules are said to be capable of specifically hybridizing to one another if the two molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure.

A nucleic acid molecule is said to be the "complement" of another nucleic acid molecule if they exhibit complete complementarity. As used herein, molecules are said to exhibit "complete complementarity" when every nucleotide of one of the molecules is complementary to a nucleotide of the other. Two molecules are said to be "minimally complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low-stringency" conditions. Similarly, the molecules are said to be "complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under conventional "high-stringency" conditions. Conventional stringency conditions are described by Sambrook *et al., Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Press*, Cold Spring Harbor, New York (1989), and by Haymes *et al., Nucleic Acid Hybridization, A Practical Approach, IRL Press*, Washington, DC (1985). Departures from complete complementarity are therefore permissible, as long as such departures do not completely preclude the capacity of the molecules to form a double-stranded structure. Thus, in order for a nucleic acid molecule to serve as a primer or probe it need only be sufficiently complementary in sequence to be able to form a stable double-stranded structure under the particular solvent and salt concentrations employed.

Appropriate stringency conditions which promote DNA hybridization are, for example, 6.0 X sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 X SSC at 20-25°C, are known to those skilled in the art or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0 X SSC at 50°C to a high stringency of about 0.2 X SSC at 65°C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22°C, to high stringency conditions at about 65°C. Both temp-

erature and salt may be varied, or either the temperature or the salt concentration may be held constant while the other variable is changed.

In a preferred embodiment, a nucleic acid of the present invention will specifically hybridize to one or more of the nucleic acid molecules set forth in SEQ ID NOs: 2-17, 50, and 85 and complements thereof under moderately stringent conditions, for example at about 2.0 X SSC and about 65°C.

In a particularly preferred embodiment, a nucleic acid of the present invention will include those nucleic acid molecules that specifically hybridize to one or more of the nucleic acid molecules set forth in SEQ ID NOs: 2-17, 50, and 85 and complements thereof under high stringency conditions such as 0.2 X SSC and about 65°C.

In one aspect of the present invention, the nucleic acid molecules of the present invention have one or more of the nucleic acid sequences set forth in SEQ ID NOs: 2-17, 50, and 85 and complements thereof. In another aspect of the present invention, one or more of the nucleic acid molecules of the present invention share between 100% and 90% sequence identity with one or more of the nucleic acid sequences set forth in SEQ ID NOs: 2-17, 50, and 85 and complements thereof and fragments of either. In a further aspect of the present invention, one or more of the nucleic acid molecules of the present invention share between 100% and 95% sequence identity with one or more of the nucleic acid sequences set forth in SEQ ID NOs: 2-17, 50, and 85, complements thereof, and fragments of either. In a more preferred aspect of the present invention, one or more of the nucleic acid molecules of the present invention share between 100% and 98% sequence identity with one or more of the nucleic acid sequences set forth in SEQ ID NOs: 2-17, 50, and 85, complements thereof and fragments of either. In an even more preferred aspect of the present invention, one or more of the nucleic acid molecules of the present invention share between 100% and 99% sequence identity with one or more of the sequences set forth in SEQ ID NOs: 2-17, 50, and 85, complements thereof, and fragments of either.

In a preferred embodiment the percent identity calculations are performed using BLASTN or BLASTP (default, parameters, version 2.0.8, Altschul *et al., Nucleic Acids Res. 25:* 3389-3402 (1997)).

A nucleic acid molecule of the invention can also encode a homolog polypeptide. As used herein, a homolog polypeptide molecule or fragment thereof is a counterpart protein molecule or fragment thereof in a second species (*e.g.*, corn rubisco small subunit

is a homolog of *Arabidopsis* rubisco small subunit). A homolog can also be generated by molecular evolution or DNA shuffling techniques, so that the molecule retains at least one functional or structure characteristic of the original polypeptide (*see*, for example, U.S. Patent 5,811,238).

In another embodiment, the homolog is selected from the group consisting of alfalfa, *Arabidopsis*, barley, *Brassica campestris,* oilseed rape, broccoli, cabbage, canola, citrus, cotton, garlic, oat, onion, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus,* crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm. More particularly, preferred homologs are selected from canola, corn, *Brassica campestris,* oilseed rape, soybean, crambe, mustard, castor bean, peanut, sesame, cottonseed, linseed, rapeseed, safflower, oil palm, flax, and sunflower. In an even more preferred embodiment, the homolog is selected from the group consisting of canola, rapeseed, corn, *Brassica campestris, Brassica napus*, soybean, sunflower, safflower, oil palms, and peanut. In a particularly preferred embodiment, the homolog is soybean. In a particularly preferred embodiment, the homolog is canola. In a particularly preferred embodiment, the homolog is *Brassica napus*.

In another further aspect of the present invention, nucleic acid molecules of the present invention can comprise sequences that differ from those encoding a polypeptide or fragment thereof in SEQ ID NOs: 19-31 and 33-38 due to the fact that a polypeptide can have one or more conservative amino acid changes, and nucleic acid sequences coding for the polypeptide can therefore have sequence differences. It is understood that codons capable of coding for such conservative amino acid substitutions are known in the art.

It is well known in the art that one or more amino acids in a native sequence can be substituted with other amino acid(s), the charge and polarity of which are similar to that of the native amino acid, *i.e.*, a conservative amino acid substitution. Conservative substitutes for an amino acid within the native polypeptide sequence can be selected from other members of the class to which the amino acid belongs. Amino acids can be divided into the following four groups: (1) acidic amino acids, (2) basic amino acids, (3) neutral polar amino acids, and (4) neutral, nonpolar amino acids. Representative amino acids within these various groups include, but are not limited to, (1) acidic (nega-

tively charged) amino acids such as aspartic acid and glutamic acid; (2) basic (positively charged) amino acids such as arginine, histidine, and lysine; (3) neutral polar amino acids such as glycine, serine, threonine, cysteine, cystine, tyrosine, asparagine, and glutamine; and (4) neutral nonpolar (hydrophobic) amino acids such as alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine.

Conservative amino acid substitution within the native polypeptide sequence can be made by replacing one amino acid from within one of these groups with another amino acid from within the same group. In a preferred aspect, biologically functional equivalents of the proteins or fragments thereof of the present invention can have ten or fewer conservative amino acid changes, more preferably seven or fewer conservative amino acid changes, and most preferably five or fewer conservative amino acid changes. The encoding nucleotide sequence will thus have corresponding base substitutions, permitting it to encode biologically functional equivalent forms of the polypeptides of the present invention.

It is understood that certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Because it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence and, of course, its underlying DNA coding sequence and, nevertheless, a protein with like properties can still be obtained. It is thus contemplated by the inventors that various changes may be made in the peptide sequences of the proteins or fragments of the present invention, or corresponding DNA sequences that encode said peptides, without appreciable loss of their biological utility or activity. It is understood that codons capable of coding for such amino acid changes are known in the art.

In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is generally understood in the art (Kyte and Doolittle, *J. Mol. Biol. 157*, 105-132 (1982)). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant polypeptide, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like.

Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics (Kyte and Doolittle, *J. Mol. Biol. 157:*105-132 (1982)); these are isoleucine (+4.5), valine (+4.2), leucine (+3.8), phenylalanine (+2.8), cysteine/cystine (+2.5), methionine (+1.9), alanine (+1.8), glycine (-0.4), threonine (-0.7), serine (-0.8), tryptophan (-0.9), tyrosine (-1.3), proline (-1.6), histidine (-3.2), glutamate (-3.5), glutamine (-3.5), aspartate (-3.5), asparagine (-3.5), lysine (-3.9), and arginine (-4.5).

In making such changes, the substitution of amino acids whose hydropathic indices are within ±2 is preferred, those that are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent 4,554,101 states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0), lysine (+3.0), aspartate (+3.0±1), glutamate (+3.0±1), serine (+0.3), asparagine (+0.2), glutamine (+0.2), glycine (0), threonine (-0.4), proline (-0.5±1), alanine (-0.5), histidine (-0.5), cysteine (-1.0), methionine (-1.3), valine (-1.5), leucine (-1.8), isoleucine (-1.8), tyrosine (-2.3), phenylalanine (-2.5), and tryptophan (-3.4).

In making such changes, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those that are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

In a further aspect of the present invention, one or more of the nucleic acid molecules of the present invention differ in nucleic acid sequence from those for which a specific sequence is provided herein because one or more codons has been replaced with a codon that encodes a conservative substitution of the amino acid originally encoded.

Agents of the invention include nucleic acid molecules that encode at least about a contiguous 10 amino acid region of a polypeptide of the present invention, more preferably at least about a contiguous 25, 40, 50, 100, or 125 amino acid region of a polypeptide of the present invention.

In a preferred embodiment, any of the nucleic acid molecules of the present invention can be operably linked to a promoter region that functions in a plant cell to

cause the production of an mRNA molecule, where the nucleic acid molecule that is linked to the promoter is heterologous with respect to that promoter. As used herein, "heterologous" means not naturally occurring together.

PROTEIN AND PEPTIDE MOLECULES

A class of agents includes one or more of the polypeptide molecules encoded by a nucleic acid agent of the invention. A particular preferred class of proteins is that having an amino acid sequence selected from the group consisting of SEQ ID NOs: 19-31 and 33-38 and fragments thereof. Polypeptide agents may have C-terminal or N-terminal amino acid sequence extensions. One class of N-terminal extensions employed in a preferred embodiment are plastid transit peptides. When employed, plastid transit peptides can be operatively linked to the N-terminal sequence, thereby permitting the localization of the agent polypeptides to plastids. In a preferred embodiment the plastid targeting sequence is a CTP1 sequence (SEQ ID NO: 32). *See* WO 00/61771.

In a preferred aspect a protein of the present invention is targeted to a plastid using either a native transit peptide sequence or a heterologous transit peptide sequence. In the case of nucleic acid sequences corresponding to nucleic acid sequences of non-higher plant organisms such as cyanobacteria, such nucleic acid sequences can be modified to attach the coding sequence of the protein to a nucleic acid sequence of a plastid targeting peptide. Examples of cynobacterial nucleic acid sequences that can be so attached are those having amino acid sequence set forth in SEQ ID NOs: 42-45.

As used herein, the term "protein," "peptide molecule," or "polypeptide" includes any molecule that comprises five or more amino acids. It is well known in the art that protein, peptide or polypeptide molecules may undergo modification, including post-translational modifications, such as, but not limited to, disulfide bond formation, glycosylation, phosphorylation, or oligomerization. Thus, as used herein, the term "protein," "peptide molecule," or "polypeptide" includes any protein that is modified by any biological or non-biological process. The terms "amino acid" and "amino acids" refer to all naturally occurring L-amino acids. This definition is meant to include norleucine, norvaline, ornithine, homocysteine, and homoserine.

One or more of the protein or fragments thereof, peptide molecules, or polypeptide molecules may be produced via chemical synthesis, or more preferably, by expression in a suitable bacterial or eukaryotic host. Suitable methods for expression are de-

scribed by Sambrook *et al.*, In: *Molecular Cloning, A Laboratory Manual, 2nd Edition, Cold Spring Harbor Press*, Cold Spring Harbor, New York (1989) or similar texts.

A "protein fragment" is a peptide or polypeptide molecule whose amino acid sequence comprises a subset of the amino acid sequence of that protein. A protein or fragment thereof that comprises one or more additional peptide regions not derived from that protein is a "fusion" protein. Such molecules may be derivatized to contain carbohydrate or other moieties (such as keyhole limpet hemocyanin). Fusion protein or peptide molecules of the invention are preferably produced via recombinant means.

Another class of agents comprise protein, peptide molecules, or polypeptide molecules or fragments or fusions thereof comprising SEQ ID NOs: 19-31 and 33-38 and fragments thereof in which conservative, non-essential or non-relevant amino acid residues have been added, replaced or deleted. Computerized means for designing modifications in protein structure are known in the art (Dahiyat and Mayo, *Science* 278:82-87 (1997)).

A protein, peptide or polypeptide of the invention can also be a homolog protein, peptide or polypeptide. As used herein, a homolog protein, peptide or polypeptide or fragment thereof is a counterpart protein, peptide or polypeptide or fragment thereof in a second species. A homolog can also be generated by molecular evolution or DNA shuffling techniques, so that the molecule retains at least one functional or structure characteristic of the original (*see*, for example, U.S. Patent 5,811,238).

In another embodiment, the homolog is selected from the group consisting of alfalfa, *Arabidopsis*, barley, broccoli, cabbage, canola, citrus, cotton, garlic, oat, onion, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, corn, and *Phaseolus*. More particularly, preferred homologs are selected from canola, rapeseed, corn, *Brassica campestris,* oilseed rape, soybean, crambe, mustard, castor bean, peanut, sesame, cottonseed, linseed, safflower, oil palm, flax, and sunflower. In an even more preferred embodiment, the homolog is selected from the group consisting of canola, rapeseed, corn, *Brassica campestris,* oilseed rape, soybean, sunflower, safflower, oil palms, and peanut. In a preferred embodiment, the homolog is soybean. In a preferred embodiment, the homolog is canola. In a preferred embodiment, the homolog is *Brassica napus*.

In a preferred embodiment, the nucleic acid molecules of the present invention or complements and fragments of either can be utilized to obtain such homologs.

Agents of the invention include proteins and fragments thereof comprising at least about a contiguous 10 amino acid region preferably comprising at least about a contiguous 20 amino acid region, even more preferably comprising at least about a contiguous 25, 35, 50, 75 or 100 amino acid region of a protein of the present invention. In another preferred embodiment, the proteins of the present invention include between about 10 and about 25 contiguous amino acid region, more preferably between about 20 and about 50 contiguous amino acid region, and even more preferably between about 40 and about 80 contiguous amino acid region.

PLANT CONSTRUCTS AND PLANT TRANSFORMANTS

One or more of the nucleic acid molecules of the invention may be used in plant transformation or transfection. Exogenous genetic material may be transferred into a plant cell and the plant cell regenerated into a whole, fertile or sterile plant. Exogenous genetic material is any genetic material, whether naturally occurring or otherwise, from any source that is capable of being inserted into any organism.

In a preferred aspect of the present invention the exogenous genetic material comprises a nucleic acid sequence that encodes a gamma-tocopherol methyltransferase. In a particularly preferred embodiment of the present invention, the exogenous genetic material of the invention comprises a nucleic acid sequence of SEQ ID NO: 2. In a further aspect of the present invention the exogenous genetic material comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 19-31, 33-38, 39-41, and 46-49 and fragments thereof.

In another preferred aspect of the present invention the exogenous genetic material comprises a nucleic acid sequence that encodes a 2-methyl-6-phytylplastoquinol/ 2-methyl-6-solanylplastoquinol-9 methyltransferase. In another preferred aspect of the present invention the exogenous genetic material of the invention comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 42-45 and complements thereof and fragments of either. In a further aspect of the present invention the exogenous genetic material comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 46-49 and fragments thereof. In a further aspect of the present invention, the nucleic acid sequences of the

invention also encode peptides involved in intracellular localization, export, or post-translational modification.

In an embodiment of the present invention, exogenous genetic material comprising a GMT or fragment thereof is introduced into a plant with one or more additional genes. In another embodiment of the present invention, exogenous genetic material comprising a MT1 or fragment thereof is introduced into a plant with one or more additional genes. In one embodiment, preferred combinations of genes include two or more of the following genes: *tyrA, slr1736, ATPT2, dxs, dxr, GGPPS, HPPD, GMT, MT1, tMT2, AANT1, slr 1737*, or a plant ortholog and an antisense construct for homogentisic acid dioxygenase (Kridl *et al., Seed Sci. Res.* 1:209:219 (1991); Keegstra, *Cell 56(2)*:247-53 (1989); Nawrath, *et al., Proc. Natl. Acad. Sci. U.S.A. 91*:12760-12764 (1994); Xia *et al., J. Gen. Microbiol. 138*:1309-1316 (1992); Cyanobase, www.kazusa.or.jp/cyanobase; Lois *et al., Proc. Natl. Acad. Sci. U.S.A. 95 (5)*:2105-2110 (1998); Takahashi *et al. Proc. Natl. Acad. Sci. U.S.A. 95 (17)*, 9879-9884 (1998); Norris *et al., Plant Physiol. 117*:1317-1323 (1998); Bartley and Scolnik, *Plant Physiol. 104*:1469-1470 (1994), Smith *et al., Plant J. 11: 8*3-92 (1997); WO 00/32757; WO 00/10380; Saint Guily, *et al., Plant Physiol., 100(2)*:1069-1071 (1992); Sato *et al., J. DNA Res. 7 (1)*:31-63 (2000)). In such combinations, one or more of the gene products can be directed to the plastid by the use of a plastid targeting sequence. Alternatively, one or more of the gene products can be localized in the cytoplasm. In a preferred aspect the gene products of *tyr*A and HPPD are targeted to the cytoplasm. Such genes can be introduced, for example, with the MT1 or GMT or both or fragment of either or both on a single construct, introduced on different constructs but the same transformation event or introduced into separate plants followed by one or more crosses to generate the desired combination of genes. In such combinations, a preferred promoter is a napin promoter and a preferred plastid targeting sequence is a CTP1 sequence. It is preferred that gene products are targeted to the plastid.

A particularly preferred combination that can be introduced is a nucleic acid molecule encoding a GMT polypeptide and a nucleic acid molecule encoding an MT1 polypeptide, where both polypeptides are targeted to the plastid and where one of such polypeptides is present and the other is introduced. Both nucleic acid sequences encoding such polypeptides are introduced using a single construct, or each polypeptide is introduced on separate constructs.

Another particularly preferred combination that can be introduced is a nucleic acid molecule encoding an MT1 protein and a nucleic acid molecule that results in the down regulation of a GMT protein. In such an aspect, it is preferred that the plant accumulates either γ-tocopherol or γ-tocotrienol or both.

Such genetic material may be transferred into either monocotyledons or dicotyledons including, but not limited to canola, corn, soybean, *Arabidopsis* phaseolus, peanut, alfalfa, wheat, rice, oat, sorghum, rapeseed, rye, tritordeum, millet, fescue, perennial ryegrass, sugarcane, cranberry, papaya, banana, safflower, oil palms, flax, muskmelon, apple, cucumber, dendrobium, gladiolus, chrysanthemum, liliacea, cotton, eucalyptus, sunflower, *Brassica campestris, Brassica napus*, turfgrass, sugarbeet, coffee and dioscorea (Christou, In: *Particle Bombardment for Genetic Engineering of Plants,* Biotechnology Intelligence Unit. Academic Press, San Diego, California (1996)), with canola, corn, *Brassica campestris, Brassica napus*, rapeseed, soybean, crambe, mustard, castor bean, peanut, sesame, cottonseed, linseed, safflower, oil palm, flax, and sunflower preferred, and canola, rapeseed, corn, *Brassica campestris, Brassica napus*, soybean, sunflower, safflower, oil palms, and peanut preferred. In a more preferred embodiment, the genetic material is transferred into canola. In another more preferred embodiment, the genetic material is transferred into *Brassica napus*. In another particularly preferred embodiment, the genetic material is transferred into soybean. In another particularly preferred embodiment of the present invention, the genetic material is transferred into soybean line 3244.

Transfer of a nucleic acid molecule that encodes a protein can result in expression or overexpression of that polypeptide in a transformed cell or transgenic plant. One or more of the proteins or fragments thereof encoded by nucleic acid molecules of the invention may be overexpressed in a transformed cell or transformed plant. Such expression or overexpression may be the result of transient or stable transfer of the exogenous genetic material.

In a preferred embodiment, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of tocopherols.

In a preferred embodiment, expression, or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of α-tocopherols.

In a preferred embodiment, expression, or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of γ-tocopherols.

In a preferred embodiment, reduction of the expression, expression, or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of δ-tocopherols.

In a preferred embodiment, reduction of the expression, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of tocotrienols.

In a preferred embodiment, reduction of the expression, expression, or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of α-tocotrienols.

In a preferred embodiment, reduction of the expression, expression, or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of γ-tocotrienols.

In a preferred embodiment, reduction of the expression, expression, or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of δ-tocotrienols.

In another embodiment, reduction of the expression, expression, overexpression of a polypeptide of the present invention in a plant provides in that plant, or a tissue of that plant, relative to an untransformed plant or plant tissue, with a similar genetic background, an increased level of an MT1 or GMT protein or both or fragment of either.

In some embodiments, the levels of one or more products of the tocopherol biosynthesis pathway, including any one or more of tocopherols, α-tocopherols, γ-tocopherols, δ-tocopherols, β-tocopherols, tocotrienols, α-tocotrienols, γ-tocotrienols, δ-tocotrienols, β-tocotrienols, are increased by greater than about 10%, or more preferably greater than about 25%, 50%, 200%, 1,000%, 2,000%, 2,500% or 25,000%. The levels of products may be increased throughout an organism such as a plant or localized in one or

more specific organs or tissues of the organism. For example the levels of products may be increased in one or more of the tissues and organs of a plant including without limitation: roots, tubers, stems, leaves, stalks, fruit, berries, nuts, bark, pods, seeds and flowers. A preferred organ is a seed.

In some embodiments, the levels of tocopherols or a species such as α-tocopherol may be altered. In some embodiments, the levels of tocotrienols may be altered. Such alteration can be compared to a plant with a similar background.

In another embodiment, either the α-tocopherol level, α-tocotrienol level, or both of plants that natively produce high levels of either α-tocopherol, α-tocotrienol or both (*e.g.*, sunflowers), can be increased by the introduction of a gene coding for an MT1 enzyme.

In a preferred aspect, a similar genetic background is a background where the organisms being compared share about 50% or greater of their nuclear genetic material. In a more preferred aspect a similar genetic background is a background where the organisms being compared share about 75% or greater, even more preferably about 90% or greater of their nuclear genetic material. In another even more preferable aspect, a similar genetic background is a background where the organisms being compared are plants, and the plants are isogenic except for any genetic material originally introduced using plant transformation techniques.

In another preferred embodiment, reduction of the expression, expression, overexpression of a polypeptide of the present invention in a transformed plant may provide tolerance to a variety of stress, *e.g.* oxidative stress tolerance such as to oxygen or ozone, UV tolerance, cold tolerance, or fungal/microbial pathogen tolerance.

As used herein in a preferred aspect, a tolerance or resistance to stress is determined by the ability of a plant, when challenged by a stress such as cold to produce a plant having a higher yield than one without such tolerance or resistance to stress. In a particularly preferred aspect of the present invention, the tolerance or resistance to stress is measured relative to a plant with a similar genetic background to the tolerant or resistance plant except that the plant reduces the expression, expresses or over expresses a protein or fragment thereof of the present invention.

Exogenous genetic material may be transferred into a host cell by the use of a DNA vector or construct designed for such a purpose. Design of such a vector is gen-

erally within the skill of the art (*See, Plant Molecular Biology: A Laboratory Manual*, Clark (ed.), Springer, New York (1997)).

A construct or vector may include a plant promoter to express the polypeptide of choice. In a preferred embodiment, any nucleic acid molecules described herein can be operably linked to a promoter region which functions in a plant cell to cause the production of an mRNA molecule. For example, any promoter that functions in a plant cell to cause the production of an mRNA molecule, such as those promoters described herein, without limitation, can be used. In a preferred embodiment, the promoter is a plant promoter.

A number of promoters that are active in plant cells have been described in the literature. These include the nopaline synthase (NOS) promoter (Ebert *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 84*:5745-5749 (1987)), the octopine synthase (OCS) promoter (which is carried on tumor-inducing plasmids of *Agrobacterium tumefaciens*), the caulimovirus promoters such as the cauliflower mosaic virus (CaMV) 19S promoter (Lawton *et al.*, *Plant Mol. Biol. 9*:315-324 (1987)) and the CaMV 35S promoter (Odell *et al.*, *Nature 313*:810-812 (1985)), the figwort mosaic virus 35S-promoter, the light-inducible promoter from the small subunit of ribulose-1,5-bis-phosphate carboxylase (ssRUBISCO), the Adh promoter (Walker *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 84*:6624-6628 (1987)), the sucrose synthase promoter (Yang *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 87*:4144-4148 (1990)), the R gene complex promoter (Chandler *et al.*, *The Plant Cell 1*:1175-1183 (1989)) and the chlorophyll a/b binding protein gene promoter, *etc.* These promoters have been used to create DNA constructs that have been expressed in plants; *see, e.g.*, PCT publication WO 84/02913. The CaMV 35S promoters are preferred for use in plants. Promoters known or found to cause transcription of DNA in plant cells can be used in the invention.

For the purpose of expression in source tissues of the plant, such as the leaf, seed, root or stem, it is preferred that the promoters utilized have relatively high expression in these specific tissues. Tissue-specific expression of a protein of the present invention is a particularly preferred embodiment. For this purpose, one may choose from a number of promoters for genes with tissue- or cell-specific or enhanced expression. Examples of such promoters reported in the literature include the chloroplast glutamine synthetase GS2 promoter from pea (Edwards *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 87*:3459-3463 (1990)), the chloroplast fructose-1,6-biphosphatase (FBPase) promoter from wheat

(Lloyd *et al.*, *Mol. Gen. Genet. 225*:209-216 (1991)), the nuclear photosynthetic ST-LS1 promoter from potato (Stockhaus *et al.*, *EMBO J. 8*:2445-2451 (1989)), the serine/threonine kinase (PAL) promoter and the glucoamylase (CHS) promoter from *Arabidopsis thaliana*. Also reported to be active in photosynthetically active tissues are the ribulose-1,5-bisphosphate carboxylase (RbcS) promoter from eastern larch (*Larix laricina*), the promoter for the *cab* gene, cab6, from pine (Yamamoto *et al.*, *Plant Cell Physiol. 35*:773-778 (1994)), the promoter for the Cab-1 gene from wheat (Fejes *et al.*, *Plant Mol. Biol. 15*:921-932 (1990)), the promoter for the CAB-1 gene from spinach (Lubberstedt *et al.*, *Plant Physiol. 104*:997-1006 (1994)), the promoter for the cab1R gene from rice (Luan *et al.*, *Plant Cell. 4*:971-981 (1992)), the pyruvate, orthophosphate dikinase (PPDK) promoter from corn (Matsuoka *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 90*: 9586-9590 (1993)), the promoter for the tobacco Lhcb1*2 gene (Cerdan *et al.*, *Plant Mol. Biol. 33*:245-255 (1997)), the *Arabidopsis thaliana* SUC2 sucrose-H+ symporter promoter (Truernit *et al.*, *Planta. 196*:564-570 (1995)) and the promoter for the thylakoid membrane proteins from spinach (*psa*D, *psa*F, *psa*E, PC, FNR, *atp*C, *atp*D, *cab*, *rbc*S). Other promoters for the chlorophyll a/b-binding proteins may also be utilized in the invention, such as the promoters for *Lhc*B gene and *Psb*P gene from white mustard (*Sinapis alba*; Kretsch *et al.*, *Plant Mol. Biol. 28*:219-229 (1995)).

For the purpose of expression in sink tissues of the plant, such as the tuber of the potato plant, the fruit of tomato, or the seed of corn, wheat, rice and barley, it is preferred that the promoters utilized in the invention have relatively high expression in these specific tissues. A number of promoters for genes with tuber-specific or tuber-enhanced expression are known, including the class I patatin promoter (Bevan *et al.*, *EMBO J. 8*:1899-1906 (1986); Jefferson *et al.*, *Plant Mol. Biol. 14*:995-1006 (1990)), the promoter for the potato tuber ADPGPP genes, both the large and small subunits, the sucrose synthase promoter (Salanoubat and Belliard, *Gene 60*:47-56 (1987), Salanoubat and Belliard, *Gene 84*:181-185 (1989)), the promoter for the major tuber proteins including the 22 kd protein complexes and protease inhibitors (Hannapel, *Plant Physiol. 101*:703-704 (1993)), the promoter for the granule-bound starch synthase gene (GBSS) (Visser *et al.*, *Plant Mol. Biol. 17*:691-699 (1991)) and other class I and II patatins promoters (Koster-Topfer *et al.*, *Mol. Gen. Genet. 219*:390-396 (1989); Mignery *et al.*, *Gene. 62*:27-44 (1988)).

Other promoters can also be used to express a polypeptide in specific tissues, such as seeds or fruits. Indeed, in a preferred embodiment, the promoter used is a seed specific promoter. Examples of such promoters include the 5' regulatory regions from such genes as napin (Kridl *et al., Seed Sci. Res.* 1:209:219 (1991)), phaseolin (Bustos, *et al., Plant Cell,* 1(9):839-853 (1989)), soybean trypsin inhibitor (Riggs, *et al., Plant Cell* 1(6):609-621 (1989)), ACP (Baerson, *et al., Plant Mol. Biol.,* 22(2):255-267 (1993)), stearoyl-ACP desaturase (Slocombe, *et al., Plant Physiol.* 104(4):167-176 (1994)), soybean α' subunit of β-conglycinin (soy 7s, (Chen *et al., Proc. Natl. Acad. Sci.,* 83:8560-8564 (1986))), and oleosin (see, for example, Hong, *et al., Plant Mol. Biol.,* 34(3):549-555 (1997)). Further examples include the promoter for β-conglycinin (Chen *et al., Dev. Genet. 10*: 112-122 (1989)). Also included are the zeins, which are a group of storage proteins found in corn endosperm. Genomic clones for zein genes have been isolated (Pedersen *et al., Cell 29*:1015-1026 (1982), and Russell *et al., Transgenic Res. 6*(2):157-168) and the promoters from these clones, including the 15 kD, 16 kD, 19 kD, 22 kD, 27 kD and genes, could also be used. Other promoters known to function, for example, in corn include the promoters for the following genes: *waxy, Brittle, Shrunken 2*, Branching enzymes I and II, starch synthases, debranching enzymes, oleosins, glutelins and sucrose synthases. A particularly preferred promoter for corn endosperm expression is the promoter for the glutelin gene from rice, more particularly the Osgt-1 promoter (Zheng *et al., Mol. Cell Biol. 13*:5829-5842 (1993)). Examples of promoters suitable for expression in wheat include those promoters for the ADPglucose pyrosynthase (ADPGPP) subunits, the granule bound and other starch synthase, the branching and debranching enzymes, the embryogenesis-abundant proteins, the gliadins and the glutenins. Examples of such promoters in rice include those promoters for the ADPGPP subunits, the granule bound and other starch synthase, the branching enzymes, the debranching enzymes, sucrose synthases and the glutelins. A particularly preferred promoter is the promoter for rice glutelin, Osgt-1. Examples of such promoters for barley include those for the ADPGPP subunits, the granule bound and other starch synthase, the branching enzymes, the debranching enzymes, sucrose synthases, the hordeins, the embryo globulins and the aleurone specific proteins. A preferred promoter for expression in the seed is a napin promoter. Another preferred promoter for expression is an Arcelin 5 promoter.

Root specific promoters may also be used. An example of such a promoter is the promoter for the acid chitinase gene (Samac *et al., Plant Mol. Biol. 25*:587-596 (1994)).

Expression in root tissue could also be accomplished by utilizing the root specific subdomains of the CaMV35S promoter that have been identified (Lam *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 86*:7890-7894 (1989)). Other root cell specific promoters include those reported by Conkling *et al.* (Conkling *et al.*, *Plant Physiol. 93*:1203-1211 (1990)).

Additional promoters that may be utilized are described, for example, in U.S. Patents 5,378,619; 5,391,725; 5,428,147; 5,447,858; 5,608,144; 5,608,144; 5,614,399; 5,633,441; 5,633,435; and 4,633,436. In addition, a tissue specific enhancer may be used (Fromm *et al.*, *The Plant Cell 1*:977-984 (1989)).

In a preferred embodiment of the invention, a nucleic acid molecule having a sequence encoding either a GMT or an MT1 enzyme is linked to a P7 or Arcelin 5 promoter. In a particularly preferred embodiment of the present invention, the promoter comprises a nucleic acid molecule having a sequence selected from the group consisting of SEQ ID NOs 81 and 82. In a particularly preferred embodiment, the invention relates to a soybean line 3244 plant, comprising an exogenous nucleic acid molecule comprising a nucleic acid sequence selected of SEQ ID NO: 2, operably linked to a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 81 and 82.

Constructs or vectors may also include, with the coding region of interest, a nucleic acid sequence that acts, in whole or in part, to terminate transcription of that region. A number of such sequences have been isolated, including the Tr7 3' sequence and the NOS 3' sequence (Ingelbrecht *et al.*, *The Plant Cell 1*:671-680 (1989); Bevan *et al.*, *Nucleic Acids Res. 11*:369-385 (1983)). Regulatory transcript termination regions can be provided in plant expression constructs of this invention as well. Transcript termination regions can be provided by the DNA sequence encoding the gene of interest or a convenient transcription termination region derived from a different gene source, for example, the transcript termination region that is naturally associated with the transcript initiation region. The skilled artisan will recognize that any convenient transcript termination region that is capable of terminating transcription in a plant cell can be employed in the constructs of the present invention.

A vector or construct may also include regulatory elements. Examples of such include the Adh intron 1 (Callis *et al.*, *Genes and Develop. 1*:1183-1200 (1987)), the sucrose synthase intron (Vasil *et al.*, *Plant Physiol. 91*:1575-1579 (1989)) and the TMV

omega element (Gallie *et al.*, *The Plant Cell 1*:301-311 (1989)). These and other regulatory elements may be included when appropriate.

A vector or construct may also include a selectable marker. Selectable markers may also be used to select for plants or plant cells that contain the exogenous genetic material. Examples of such include, but are not limited to: a *neo* gene (Potrykus *et al.*, *Mol. Gen. Genet. 199*:183-188 (1985)), which codes for kanamycin resistance and can be selected for using kanamycin, RptII, G418, hpt *etc.*; a bar gene which codes for bialaphos resistance; a mutant EPSP synthase gene (Hinchee *et al.*, *Bio/Technology 6*:915-922 (1988); Reynaerts *et al.*, Selectable and Screenable Markers. In Gelvin and Schilperoort. Plant Molecular Biology Manual, Kluwer, Dordrecht (1988); Reynaerts *et al.*, Selectable and screenable markers. In Gelvin and Schilperoort. Plant Molecular Biology Manual, Kluwer, Dordrecht (1988)), *aad*A (Jones *et al.*, *Mol. Gen. Genet.* (1987)),) which encodes glyphosate resistance; a nitrilase gene which confers resistance to bromoxynil (Stalker *et al.*, *J. Biol. Chem. 263*:6310-6314 (1988)); a mutant acetolactate synthase gene (ALS) which confers imidazolinone or sulphonylurea resistance (European Patent Application 154,204 (Sept. 11, 1985)), ALS (D'Halluin *et al.*, Bio/Technology 10: 309-314 (1992)), and a methotrexate resistant DHFR gene (Thillet *et al.*, *J. Biol. Chem. 263*:12500-12508 (1988)).

A vector or construct may also include a transit peptide. Incorporation of a suitable chloroplast transit peptide may also be employed (European Patent Application Publication Number 0218571). Translational enhancers may also be incorporated as part of the vector DNA. DNA constructs could contain one or more 5' non-translated leader sequences, which may serve to enhance expression of the gene products from the resulting mRNA transcripts. Such sequences may be derived from the promoter selected to express the gene or can be specifically modified to increase translation of the mRNA. Such regions may also be obtained from viral RNAs, from suitable eukaryotic genes, or from a synthetic gene sequence. For a review of optimizing expression of transgenes, see Koziel *et al.*, *Plant Mol. Biol. 32*:393-405 (1996). A preferred transit peptide is CTP1.

A vector or construct may also include a screenable marker. Screenable markers may be used to monitor expression. Exemplary screenable markers include: a β-glucuronidase or *uid*A gene (GUS) which encodes an enzyme for which various chromogenic substrates are known (Jefferson, *Plant Mol. Biol, Rep. 5*:387-405 (1987); Jefferson *et al.*, *EMBO J. 6*:3901-3907 (1987)); an R-locus gene, which encodes a product that regulates

the production of anthocyanin pigments (red color) in plant tissues (Dellaporta *et al.*, Stadler Symposium *11*:263-282 (1988)); a β-lactamase gene (Sutcliffe *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 75*:3737-3741 (1978)), a gene which encodes an enzyme for which various chromogenic substrates are known (*e.g.*, PADAC, a chromogenic cephalosporin); a luciferase gene (Ow *et al.*, *Science 234*:856-859 (1986)); a *xyl*E gene (Zukowsky *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 80*:1101-1105 (1983)) which encodes a catechol dioxygenase that can convert chromogenic catechols; an α-amylase gene (Ikatu *et al.*, *Bio/Technol. 8*:241-242 (1990)); a tyrosinase gene (Katz *et al.*, *J. Gen. Microbiol. 129*:2703-2714 (1983)) which encodes an enzyme capable of oxidizing tyrosine to DOPA and dopaquinone which in turn condenses to melanin; an α-galactosidase, which will turn a chromogenic α-galactose substrate.

Included within the terms "selectable or screenable marker genes" are also genes that encode a secretable marker whose secretion can be detected as a means of identifying or selecting for transformed cells. Examples include markers that encode a secretable antigen that can be identified by antibody interaction, or even secretable enzymes that can be detected catalytically. Secretable proteins fall into a number of classes, including small, diffusible proteins that are detectable, (*e.g.*, by ELISA), small active enzymes that are detectable in extracellular solution (*e.g.*, α-amylase, β-lactamase, phosphinothricin transferase), or proteins that are inserted or trapped in the cell wall (such as proteins that include a leader sequence such as that found in the expression unit of extension or tobacco PR-S). Other possible selectable and/or screenable marker genes will be apparent to those of skill in the art.

There are many methods for introducing transforming nucleic acid molecules into plant cells. Suitable methods are believed to include virtually any method by which nucleic acid molecules may be introduced into a cell, such as by *Agrobacterium* infection or direct delivery of nucleic acid molecules such as, for example, by PEG-mediated transformation, by electroporation or by acceleration of DNA coated particles, and the like. (Potrykus, *Ann. Rev. Plant Physiol. Plant Mol. Biol. 42*:205-225 (1991); Vasil, *Plant Mol. Biol. 25*:925-937 (1994)). For example, electroporation has been used to transform corn protoplasts (Fromm *et al.*, *Nature 312*:791-793 (1986)).

Other vector systems suitable for introducing transforming DNA into a host plant cell include but are not limited to binary artificial chromosome (BIBAC) vectors (Hamilton *et al.*, *Gene 200*:107-116 (1997)); and transfection with RNA viral vectors (Della-

Cioppa *et al., Ann. N.Y. Acad. Sci.* (1996), 792 (Engineering Plants for Commercial Products and Applications), 57-61). Additional vector systems also include plant selectable YAC vectors such as those described in Mullen *et al., Molecular Breeding 4*:449-457 (1988).

Technology for introduction of DNA into cells is well known to those of skill in the art. Four general methods for delivering a gene into cells have been described: (1) chemical methods (Graham and van der Eb, *Virology* 54:536-539 (1973)); (2) physical methods such as microinjection (Capecchi, *Cell* 22:479-488 (1980)), electroporation (Wong and Neumann, *Biochem. Biophys. Res. Commun. 107*:584-587 (1982); Fromm *et al., Proc. Natl. Acad. Sci. (U.S.A.) 82*:5824-5828 (1985); U.S. Patent 5,384,253); the gene gun (Johnston and Tang, *Methods Cell Biol. 43*:353-365 (1994)); and vacuum infiltration (Bechtold *et al., C.R. Acad. Sci. Paris, Life Sci. 316*:1194-1199. (1993)); (3) viral vectors (Clapp, *Clin. Perinatol. 20*:155-168 (1993); Lu *et al., J. Exp. Med. 178*:2089-2096 (1993); Eglitis and Anderson, *Biotechniques 6*:608-614 (1988)); and (4) receptor-mediated mechanisms (Curiel *et al., Hum. Gen. Ther. 3*:147-154 (1992), Wagner *et al., Proc. Natl. Acad. Sci. (USA) 89*:6099-6103 (1992)).

Acceleration methods that may be used include, for example, microprojectile bombardment and the like. One example of a method for delivering transforming nucleic acid molecules into plant cells is microprojectile bombardment. This method has been reviewed by Yang and Christou (eds.), *Particle Bombardment Technology for Gene Transfer*, Oxford Press, Oxford, England (1994)). Non-biological particles (microprojectiles) may be coated with nucleic acids and delivered into cells by a propelling force. Exemplary particles include those comprised of tungsten, gold, platinum and the like.

A particular advantage of microprojectile bombardment, in addition to it being an effective means of reproducibly transforming monocots, is that neither the isolation of protoplasts (Cristou *et al., Plant Physiol. 87*:671-674 (1988)) nor the susceptibility to *Agrobacterium* infection is required. An illustrative embodiment of a method for delivering DNA into corn cells by acceleration is a biolistics α-particle delivery system, which can be used to propel particles coated with DNA through a screen, such as a stainless steel or Nytex screen, onto a filter surface covered with corn cells cultured in suspension. Gordon-Kamm *et al.,* describes the basic procedure for coating tungsten particles with DNA (Gordon-Kamm *et al., Plant Cell 2*:603-618 (1990)). The screen disperses the tungsten nucleic acid particles so that they are not delivered to the recipient

cells in large aggregates. A particle delivery system suitable for use with the invention is the helium acceleration PDS-1000/He gun, which is available from Bio-Rad Laboratories (Bio-Rad, Hercules, California)(Sanford *et al., Technique 3*:3-16 (1991)).

For the bombardment, cells in suspension may be concentrated on filters. Filters containing the cells to be bombarded are positioned at an appropriate distance below the microprojectile stopping plate. If desired, one or more screens are also positioned between the gun and the cells to be bombarded.

Alternatively, immature embryos or other target cells may be arranged on solid culture medium. The cells to be bombarded are positioned at an appropriate distance below the microprojectile stopping plate. If desired, one or more screens are also positioned between the acceleration device and the cells to be bombarded. Through the use of techniques set forth herein one may obtain 1000 or more loci of cells transiently expressing a marker gene. The number of cells in a focus that express the exogenous gene product 48 hours post-bombardment often ranges from one to ten, and average one to three.

In bombardment transformation, one may optimize the pre-bombardment culturing conditions and the bombardment parameters to yield the maximum numbers of stable transformants. Both the physical and biological parameters for bombardment are important in this technology. Physical factors are those that involve manipulating the DNA/microprojectile precipitate or those that affect the flight and velocity of either the macro- or microprojectiles. Biological factors include all steps involved in manipulation of cells before and immediately after bombardment, the osmotic adjustment of target cells to help alleviate the trauma associated with bombardment and also the nature of the transforming DNA, such as linearized DNA or intact supercoiled plasmids. It is believed that pre-bombardment manipulations are especially important for successful transformation of immature embryos.

In another alternative embodiment, plastids can be stably transformed. Methods disclosed for plastid transformation in higher plants include the particle gun delivery of DNA containing a selectable marker and targeting of the DNA to the plastid genome through homologous recombination (Svab *et al., Proc. Natl. Acad. Sci. (U.S.A.) 87*:8526-8530 (1990); Svab and Maliga, *Proc. Natl. Acad. Sci. (U.S.A.) 90:*913-917 (1993); Staub and Maliga, *EMBO J. 12:*601-606 (1993); U.S. Patents 5, 451,513 and 5,545,818).

Accordingly, it is contemplated that one may wish to adjust various aspects of the bombardment parameters in small scale studies to fully optimize the conditions. One may particularly wish to adjust physical parameters such as gap distance, flight distance, tissue distance and helium pressure. One may also minimize the trauma reduction factors by modifying conditions that influence the physiological state of the recipient cells and which may therefore influence transformation and integration efficiencies. For example, the osmotic state, tissue hydration and the subculture stage or cell cycle of the recipient cells may be adjusted for optimum transformation. The execution of other routine adjustments will be known to those of skill in the art in light of the present disclosure.

Agrobacterium-mediated transfer is a widely applicable system for introducing genes into plant cells because the DNA can be introduced into whole plant tissues, thereby bypassing the need for regeneration of an intact plant from a protoplast. The use of *Agrobacterium*-mediated plant integrating vectors to introduce DNA into plant cells is well known in the art. See, for example the methods described by Fraley *et al., Bio/Technology 3*:629-635 (1985) and Rogers *et al., Methods Enzymol. 153*:253-277 (1987). Further, the integration of the Ti-DNA is a relatively precise process resulting in few rearrangements. The region of DNA to be transferred is defined by the border sequences and intervening DNA is usually inserted into the plant genome as described (Spielmann *et al., Mol. Gen. Genet. 205*:34 (1986)).

Modern *Agrobacterium* transformation vectors are capable of replication in *E. coli* as well as *Agrobacterium*, allowing for convenient manipulations as described (Klee *et al.*, In: *Plant DNA Infectious Agents*, Hohn and Schell (eds.), Springer-Verlag, New York, pp. 179-203 (1985)). Moreover, technological advances in vectors for *Agrobacterium*-mediated gene transfer have improved the arrangement of genes and restriction sites in the vectors to facilitate construction of vectors capable of expressing various polypeptide coding genes. The vectors described have convenient multi-linker regions flanked by a promoter and a polyadenylation site for direct expression of inserted polypeptide coding genes and are suitable for present purposes (Rogers *et al., Methods Enzymol. 153*:253-277 (1987)). In addition, *Agrobacterium* containing both armed and disarmed Ti genes can be used for the transformations. In those plant strains where *Agrobacterium*-mediated transformation is efficient, it is the method of choice because of the facile and defined nature of the gene transfer.

A transgenic plant formed using *Agrobacterium* transformation methods typically contains a single gene on one chromosome. Such transgenic plants can be referred to as being heterozygous for the added gene. More preferred is a transgenic plant that is homozygous for the added structural gene; *i.e.*, a transgenic plant that contains two added genes, one gene at the same locus on each chromosome of a chromosome pair. A homozygous transgenic plant can be obtained by sexually mating (selfing) an independent segregant, transgenic plant that contains a single added gene, germinating some of the seed produced and analyzing the resulting plants produced for the gene of interest.

It is also to be understood that two different transgenic plants can also be mated to produce offspring that contain two independently segregating, exogenous genes. Selfing of appropriate progeny can produce plants that are homozygous for both added, exogenous genes that encode a polypeptide of interest. Back-crossing to a parental plant and out-crossing with a non-transgenic plant are also contemplated, as is vegetative propagation.

Transformation of plant protoplasts can be achieved using methods based on calcium phosphate precipitation, polyethylene glycol treatment, electroporation and combinations of these treatments (*See, for example*, Potrykus *et al., Mol. Gen. Genet. 205*:193-200 (1986); Lorz *et al., Mol. Gen. Genet. 199*:178 (1985); Fromm *et al., Nature 319*:791 (1986); Uchimiya *et al., Mol. Gen. Genet. 204*:204 (1986); Marcotte *et al., Nature 335*:454-457 (1988)).

Application of these systems to different plant strains depends upon the ability to regenerate that particular plant strain from protoplasts. Illustrative methods for the regeneration of cereals from protoplasts are described (Fujimura *et al., Plant Tissue Culture Letters 2*:74 (1985); Toriyama *et al., Theor. Appl. Genet. 205*:34 (1986); Yamada *et al., Plant Cell Rep. 4*:85 (1986); Abdullah *et al., Biotechnology 4*:1087 (1986)).

To transform plant strains that cannot be successfully regenerated from protoplasts, other ways to introduce DNA into intact cells or tissues can be utilized. For example, regeneration of cereals from immature embryos or explants can be effected as described (Vasil, *Biotechnology 6*:397 (1988)). In addition, "particle gun" or high-velocity microprojectile technology can be utilized (Vasil *et al., Bio/Technology 10*:667 (1992)).

Using the latter technology, DNA is carried through the cell wall and into the cytoplasm on the surface of small metal particles as described (Klein *et al., Nature*

*328:*70 (1987); Klein *et al., Proc. Natl. Acad. Sci. (U.S.A.) 85*:8502-8505 (1988); McCabe *et al., Bio/Technology 6:923* (1988)). The metal particles penetrate through several layers of cells and thus allow the transformation of cells within tissue explants.

Other methods of cell transformation can also be used and include but are not limited to introduction of DNA into plants by direct DNA transfer into pollen (Hess *et al., Intern Rev. Cytol. 107:*367 (1987); Luo *et al., Plant Mol Biol. Reporter 6:*165 (1988)), by direct injection of DNA into reproductive organs of a plant (Pena *et al., Nature 325*:274 (1987)), or by direct injection of DNA into the cells of immature embryos followed by the rehydration of desiccated embryos (Neuhaus *et al., Theor. Appl. Genet. 75*:30 (1987)).

The regeneration, development and cultivation of plants from single plant protoplast transformants or from various transformed explants is well known in the art (Weissbach and Weissbach, In: *Methods for Plant Molecular Biology*, Academic Press, San Diego, CA, (1988)). This regeneration and growth process typically includes the steps of selection of transformed cells, culturing those individualized cells through the usual stages of embryonic development through the rooted plantlet stage. Transgenic embryos and seeds are similarly regenerated. The resulting transgenic rooted shoots are thereafter planted in an appropriate plant growth medium such as soil.

The development or regeneration of plants containing the foreign, exogenous gene that encodes a protein of interest is well known in the art. Preferably, the regenerated plants are self-pollinated to provide homozygous transgenic plants. Otherwise, pollen obtained from the regenerated plants is crossed to seed-grown plants of agronomically important lines. Conversely, pollen from plants of these important lines is used to pollinate regenerated plants. A transgenic plant of the invention containing a desired polypeptide is cultivated using methods well known to one skilled in the art.

There are a variety of methods for the regeneration of plants from plant tissue. The particular method of regeneration will depend on the starting plant tissue and the particular plant species to be regenerated.

Methods for transforming dicots, primarily by use of *Agrobacterium tumefaciens* and obtaining transgenic plants have been published for cotton (U.S. Patent 5,004,863; U.S. Patent 5,159,135; U.S. Patent 5,518,908); soybean (U.S. Patent 5,569,834; U.S. Patent 5,416,011; McCabe *et al., Biotechnology 6:*923 (1988); Christou *et al., Plant Physiol. 87*:671-674 (1988)); *Brassica* (U.S. Patent 5,463,174); peanut (Cheng *et al.,*

*Plant Cell Rep. 15:*653-657 (1996), McKently *et al., Plant Cell Rep. 14:*699-703 (1995)); papaya; pea (Grant *et al., Plant Cell Rep. 15*:254-258 (1995)); and *Arabidopsis thaliana* (Bechtold *et al., C.R. Acad. Sci. Paris, Life Sci. 316*:1194-1199 (1993)). The latter method for transforming *Arabidopsis thaliana* is commonly called "dipping" or vacuum infiltration or germplasm transformation.

Transformation of monocotyledons using electroporation, particle bombardment and *Agrobacterium* have also been reported. Transformation and plant regeneration have been achieved in asparagus (Bytebier *et al., Proc. Natl. Acad. Sci. (USA) 84*:5354 (1987)); barley (Wan and Lemaux, *Plant Physiol 104:*37 (1994)); corn (Rhodes *et al., Science 240:*204 (1988); Gordon-Kamm *et al., Plant Cell* 2:603-618 (1990); Fromm *et al., Bio/Technology 8*:833 (1990); Koziel *et al., Bio/Technology 11*:194 (1993); Armstrong *et al., Crop Science 35:*550-557 (1995)); oat (Somers *et al., Bio/Technology 10*:1589 (1992)); orchard grass (Horn *et al., Plant Cell Rep. 7*:469 (1988)); rice (Toriyama *et al., Theor Appl. Genet. 205*:34 (1986); Part *et al., Plant Mol. Biol. 32*:1135-1148 (1996); Abedinia *et al., Aust. J. Plant Physiol. 24:*133-141 (1997); Zhang and Wu, *Theor. Appl. Genet. 76*:835 (1988); Zhang *et al., Plant Cell Rep. 7*:379 (1988); Battraw and Hall, *Plant Sci. 86*:191-202 (1992); Christou *et al., Bio/Technology 9*:957 (1991)); rye (De la Pena *et al., Nature 325*:274 (1987)); sugarcane (Bower and Birch, *Plant J. 2*:409 (1992)); tall fescue (Wang *et al., Bio/Technology 10*:691 (1992)) and wheat (Vasil *et al., Bio/Technology 10*:667 (1992); U.S. Patent 5,631,152).

Assays for gene expression based on the transient expression of cloned nucleic acid constructs have been developed by introducing the nucleic acid molecules into plant cells by polyethylene glycol treatment, electroporation, or particle bombardment (Marcotte *et al., Nature 335*:454-457 (1988); Marcotte *et al., Plant Cell 1:*523-532 (1989); McCarty *et al., Cell 66:*895-905 (1991); Hattori *et al., Genes Dev. 6:*609-618 (1992); Goff *et al., EMBO J. 9:*2517-2522 (1990)). Transient expression systems may be used to functionally dissect gene constructs (*see generally*, Mailga *et al., Methods in Plant Molecular Biology,* Cold Spring Harbor Press (1995)).

Any of the nucleic acid molecules of the invention may be introduced into a plant cell in a permanent or transient manner in combination with other genetic elements such as vectors, promoters, enhancers, *etc.* Further, any of the nucleic acid molecules of the invention may be introduced into a plant cell in a manner that allows for expression or overexpression of the protein or fragment thereof encoded by the nucleic acid molecule.

Cosuppression is the reduction in expression levels, usually at the level of RNA, of a particular endogenous gene or gene family by the expression of a homologous sense construct that is capable of transcribing mRNA of the same strandedness as the transcript of the endogenous gene (Napoli *et al., Plant Cell 2*:279-289 (1990); van der Krol *et al., Plant Cell 2*:291-299 (1990)). Cosuppression may result from stable transformation with a single copy nucleic acid molecule that is homologous to a nucleic acid sequence found with the cell (Prolls and Meyer, *Plant J. 2:*465-475 (1992)) or with multiple copies of a nucleic acid molecule that is homologous to a nucleic acid sequence found with the cell (Mittlesten *et al., Mol. Gen. Genet. 244:*325-330 (1994)). Genes, even though different, linked to homologous promoters may result in the cosuppression of the linked genes (Vaucheret, *C.R. Acad. Sci. III 316:*1471-1483 (1993); Flavell, *Proc. Natl. Acad. Sci. (U.S.A.) 91*:3490-3496 (1994)); van Blokland *et al., Plant J. 6*:861-877 (1994); Jorgensen, *Trends Biotechnol. 8*:340-344 (1990); Meins and Kunz, In: *Gene Inactivation and Homologous Recombination in Plants*, Paszkowski (ed.), pp. 335-348, Kluwer Academic, Netherlands (1994)).

It is understood that one or more of the nucleic acids of the invention may be introduced into a plant cell and transcribed using an appropriate promoter with such transcription resulting in the cosuppression of an endogenous protein.

Antisense approaches are a way of preventing or reducing gene function by targeting the genetic material (Mol *et al., FEBS Lett. 268*:427-430 (1990)). The objective of the antisense approach is to use a sequence complementary to the target gene to block its expression and create a mutant cell line or organism in which the level of a single chosen protein is selectively reduced or abolished. Antisense techniques have several advantages over other 'reverse genetic' approaches. The site of inactivation and its developmental effect can be manipulated by the choice of promoter for antisense genes or by the timing of external application or microinjection. Antisense can manipulate its specificity by selecting either unique regions of the target gene or regions where it shares homology to other related genes (Hiatt *et al.,* In: *Genetic Engineering,* Setlow (ed.), Vol. 11, New York: Plenum 49-63 (1989)).

Antisense RNA techniques involve introduction of RNA that is complementary to the target mRNA into cells, which results in specific RNA:RNA duplexes being formed by base pairing between the antisense substrate and the target mRNA (Green *et al., Annu. Rev. Biochem. 55*:569-597 (1986)). Under one embodiment, the process in-

volves the introduction and expression of an antisense gene sequence. Such a sequence is one in which part or all of the normal gene sequences are placed under a promoter in inverted orientation so that the 'wrong' or complementary strand is transcribed into a noncoding antisense RNA that hybridizes with the target mRNA and interferes with its expression (Takayama and Inouye, *Crit. Rev. Biochem. Mol. Biol. 25*:155-184 (1990)). An antisense vector is constructed by standard procedures and introduced into cells by transformation, transfection, electroporation, microinjection, infection, *etc.* The type of transformation and choice of vector will determine whether expression is transient or stable. The promoter used for the antisense gene may influence the level, timing, tissue, specificity, or inducibility of the antisense inhibition.

It is understood that the activity of a protein in a plant cell may be reduced or depressed by growing a transformed plant cell containing a nucleic acid molecule whose non-transcribed strand encodes a protein or fragment thereof. Preferred proteins whose activity can be reduced or depressed, by any method, are MT1 and homogenistic acid dehydrogenase. In such an embodiment of the invention, it is preferred that the concentration of γ-tocopherol or γ-tocotrienol is increased.

Posttranscriptional gene silencing (PTGS) can result in virus immunity or gene silencing in plants. PTGS is induced by dsRNA and is mediated by an RNA-dependent RNA polymerase, present in the cytoplasm, which requires a dsRNA template. The dsRNA is formed by hybridization of complementary transgene mRNAs or complementary regions of the same transcript. Duplex formation can be accomplished by using transcripts from one sense gene and one antisense gene colocated in the plant genome, a single transcript that has self-complementarity, or sense and antisense transcripts from genes brought together by crossing. The dsRNA-dependent RNA polymerase makes a complementary strand from the transgene mRNA and RNAse molecules attach to this complementary strand (cRNA). These cRNA-RNase molecules hybridize to the endogene mRNA and cleave the single-stranded RNA adjacent to the hybrid. The cleaved single-stranded RNAs are further degraded by other host RNases because one will lack a capped 5' end and the other will lack a poly(A) tail (Waterhouse *et al., PNAS 95*: 13959-13964 (1998)).

It is understood that one or more of the nucleic acids of the invention may be introduced into a plant cell and transcribed using an appropriate promoter with such transcription resulting in the posttranscriptional gene silencing of an endogenous transcript.

Antibodies have been expressed in plants (Hiatt *et al., Nature 342:*76-78 (1989); Conrad and Fielder, *Plant Mol. Biol. 26:*1023-1030 (1994)). Cytoplasmic expression of a scFv (single-chain Fv antibody) has been reported to delay infection by artichoke mottled crinkle virus. Transgenic plants that express antibodies directed against endogenous proteins may exhibit a physiological effect (Philips *et al., EMBO J. 16:*4489-4496 (1997); Marion-Poll, *Trends in Plant Science 2:*447-448 (1997)). For example, expressed anti-abscisic antibodies have been reported to result in a general perturbation of seed development (Philips *et al., EMBO J. 16:* 4489-4496 (1997)).

Antibodies that are catalytic may also be expressed in plants (abzymes). The principle behind abzymes is that since antibodies may be raised against many molecules, this recognition ability can be directed toward generating antibodies that bind transition states to force a chemical reaction forward (Persidas, *Nature Biotechnology 15:*1313-1315 (1997); Baca *et al., Ann. Rev. Biophys. Biomol. Struct. 26:*461-493 (1997)). The catalytic abilities of abzymes may be enhanced by site directed mutagenesis. Examples of abzymes are, for example, set forth in U.S. Patent: 5,658,753; U.S. Patent 5,632,990; U.S. Patent 5,631,137; U.S. Patent 5,602,015; U.S. Patent 5,559,538; U.S. Patent 5,576,174; U.S. Patent 5,500,358; U.S. Patent 5,318,897; U.S. Patent 5,298,409; U.S. Patent 5,258,289 and U.S. Patent 5,194,585.

It is understood that any of the antibodies of the invention may be expressed in plants and that such expression can result in a physiological effect. It is also understood that any of the expressed antibodies may be catalytic.

The present invention also provides for parts of the plants, particularly reproductive or storage parts, of the present invention. Plant parts, without limitation, include seed, endosperm, ovule and pollen. In a particularly preferred embodiment of the present invention, the plant part is a seed. In one embodiment the seed is a constituent of animal feed.

In another embodiment, the plant part is a fruit, more preferably a fruit with enhanced shelf life. In another preferred embodiment, the fruit has increased levels of a tocopherol. In another preferred embodiment, the fruit has increased levels of a tocotrienol.

The present invention also provides a container of over about 10,000, more preferably about 20,000, and even more preferably about 40,000 seeds where over about

10%, more preferably about 25%, more preferably about 50% and even more preferably about 75% or 90% of the seeds are seeds derived from a plant of the present invention.

The present invention also provides a container of over about 10 kg, more preferably about 25 kg, and even more preferably about 50 kg seeds where over about 10%, more preferably about 25%, more preferably about 50% and even more preferably about 75% or 90% of the seeds are seeds derived from a plant of the present invention.

Any of the plants or parts thereof of the present invention may be processed to produce a feed, meal, protein or oil preparation. A particularly preferred plant part for this purpose is a seed. In a preferred embodiment the feed, meal, protein or oil preparation is designed for ruminant animals. Methods to produce feed, meal, protein and oil preparations are known in the art. See, for example, U.S. Patents 4,957,748, 5,100,679, 5,219,596, 5,936,069, 6,005,076, 6,146,669, and 6,156,227. In a preferred embodiment, the protein preparation is a high protein preparation. Such a high protein preparation preferably has a protein content of greater than 5% w/v, more preferably 10% w/v, and even more preferably 15% w/v. In a preferred oil preparation, the oil preparation is a high oil preparation with an oil content derived from a plant or part thereof of the present invention of greater than 5% w/v, more preferably 10% w/v, and even more preferably 15% w/v. In a preferred embodiment the oil preparation is a liquid and of a volume greater than 1, 5, 10 or 50 liters. The present invention provides for oil produced from plants of the present invention or generated by a method of the present invention. Such an oil may exhibit enhanced oxidative stability. Also, such oil may be a minor or major component of any resultant product. Moreover, such oil may be blended with other oils. In a preferred embodiment, the oil produced from plants of the present invention or generated by a method of the present invention constitutes greater than 0.5%, 1%, 5%, 10%, 25%, 50%, 75% or 90% by volume or weight of the oil component of any product. In another embodiment, the oil preparation may be blended and can constitute greater than 10%, 25%, 35%, 50% or 75% of the blend by volume. Oil produced from a plant of the present invention can be admixed with one or more organic solvents or petroleum distillates.

Plants of the present invention can be part of or generated from a breeding program. The choice of breeding method depends on the mode of plant reproduction, the heritability of the trait(s) being improved, and the type of cultivar used commercially (*e.g.*, F_1 hybrid cultivar, pureline cultivar, etc). Selected, non-limiting approaches, for

breeding the plants of the present invention are set forth below. A breeding program can be enhanced using marker assisted selection of the progeny of any cross. It is further understood that any commercial and non-commercial cultivars can be utilized in a breeding program. Factors such as, for example, emergence vigor, vegetative vigor, stress tolerance, disease resistance, branching, flowering, seed set, seed size, seed density, standability, and threshability etc. will generally dictate the choice.

For highly heritable traits, a choice of superior individual plants evaluated at a single location will be effective, whereas for traits with low heritability, selection should be based on mean values obtained from replicated evaluations of families of related plants. Popular selection methods commonly include pedigree selection, modified pedigree selection, mass selection, and recurrent selection. In a preferred embodiment a backcross or recurrent breeding program is undertaken.

The complexity of inheritance influences choice of the breeding method. Backcross breeding can be used to transfer one or a few favorable genes for a highly heritable trait into a desirable cultivar. This approach has been used extensively for breeding disease-resistant cultivars. Various recurrent selection techniques are used to improve quantitatively inherited traits controlled by numerous genes. The use of recurrent selection in self-pollinating crops depends on the ease of pollination, the frequency of successful hybrids from each pollination, and the number of hybrid offspring from each successful cross.

Breeding lines can be tested and compared to appropriate standards in environments representative of the commercial target area(s) for two or more generations. The best lines are candidates for new commercial cultivars; those still deficient in traits may be used as parents to produce new populations for further selection.

One method of identifying a superior plant is to observe its performance relative to other experimental plants and to a widely grown standard cultivar. If a single observation is inconclusive, replicated observations can provide a better estimate of its genetic worth. A breeder can select and cross two or more parental lines, followed by repeated selfing and selection, producing many new genetic combinations.

The development of new cultivars requires the development and selection of varieties, the crossing of these varieties and the selection of superior hybrid crosses. The hybrid seed can be produced by manual crosses between selected male-fertile parents or by using male sterility systems. Hybrids are selected for certain single gene traits such as

pod color, flower color, seed yield, pubescence color, or herbicide resistance, which indicate that the seed is truly a hybrid. Additional data on parental lines, as well as the phenotype of the hybrid, influence the breeder's decision whether to continue with the specific hybrid cross.

Pedigree breeding and recurrent selection breeding methods can be used to develop cultivars from breeding populations. Breeding programs combine desirable traits from two or more cultivars or various broad-based sources into breeding pools from which cultivars are developed by selfing and selection of desired phenotypes. New cultivars can be evaluated to determine which have commercial potential.

Pedigree breeding is used commonly for the improvement of self-pollinating crops. Two parents who possess favorable, complementary traits are crossed to produce an F_1. A F_2 population is produced by selfing one or several F_1's. Selection of the best individuals from the best families is carried out. Replicated testing of families can begin in the F_4 generation to improve the effectiveness of selection for traits with low heritability. At an advanced stage of inbreeding (*i.e.*, F_6 and F_7), the best lines or mixtures of phenotypically similar lines are tested for potential release as new cultivars.

Backcross breeding has been used to transfer genes for a simply inherited, highly heritable trait into a desirable homozygous cultivar or inbred line, which is the recurrent parent. The source of the trait to be transferred is called the donor parent. The resulting plant is expected to have the attributes of the recurrent parent (*e.g.*, cultivar) and the desirable trait transferred from the donor parent. After the initial cross, individuals possessing the phenotype of the donor parent are selected and repeatedly crossed (backcrossed) to the recurrent parent. The resulting parent is expected to have the attributes of the recurrent parent (*e.g.*, cultivar) and the desirable trait transferred from the donor parent.

The single-seed descent procedure in the strict sense refers to planting a segregating population, harvesting a sample of one seed per plant, and using the one-seed sample to plant the next generation. When the population has been advanced from the F_2 to the desired level of inbreeding, the plants from which lines are derived will each trace to different F_2 individuals. The number of plants in a population declines each generation due to failure of some seeds to germinate or some plants to produce at least one seed. As a result, not all of the F_2 plants originally sampled in the population will be represented by a progeny when generation advance is completed.

In a multiple-seed procedure, breeders commonly harvest one or more pods from each plant in a population and thresh them together to form a bulk. Part of the bulk is used to plant the next generation and part is put in reserve. The procedure has been referred to as modified single-seed descent or the pod-bulk technique.

The multiple-seed procedure has been used to save labor at harvest. It is considerably faster to thresh pods with a machine than to remove one seed from each by hand for the single-seed procedure. The multiple-seed procedure also makes it possible to plant the same number of seed of a population each generation of inbreeding.

Descriptions of other breeding methods that are commonly used for different traits and crops can be found in one of several reference books (*e.g.* Fehr, *Principles of Cultivar Development* Vol. 1, pp. 2-3 (1987))).

A transgenic plant of the present invention may also be reproduced using apomixis. Apomixis is a genetically controlled method of reproduction in plants where the embryo is formed without union of an egg and a sperm. There are three basic types of apomictic reproduction: 1) apospory where the embryo develops from a chromosomally unreduced egg in an embryo sac derived from the nucleus, 2) diplospory where the embryo develops from an unreduced egg in an embryo sac derived from the megaspore mother cell, and 3) adventitious embryony where the embryo develops directly from a somatic cell. In most forms of apomixis, pseudogamy or fertilization of the polar nuclei to produce endosperm is necessary for seed viability. In apospory, a nurse cultivar can be used as a pollen source for endosperm formation in seeds. The nurse cultivar does not affect the genetics of the aposporous apomictic cultivar since the unreduced egg of the cultivar develops parthenogenetically, but makes possible endosperm production. Apomixis is economically important, especially in transgenic plants, because it causes any genotype, no matter how heterozygous, to breed true. Thus, with apomictic reproduction, heterozygous transgenic plants can maintain their genetic fidelity throughout repeated life cycles. Methods for the production of apomictic plants are known in the art. *See*, U.S. Patent 5,811,636.

OTHER ORGANISMS

A nucleic acid of the present invention may be introduced into any cell or organism such as a mammalian cell, mammal, fish cell, fish, bird cell, bird, algae cell, algae, fungal cell, fungi, or bacterial cell. A protein of the present invention may be produced in an appropriate cell or organism. Preferred host and transformants include: fungal cells

such as *Aspergillus*, yeasts, mammals, particularly bovine and porcine, insects, bacteria, and algae. Particularly preferred bacteria are *agrobacterium tumefaciens* and *E. coli.*

Methods to transform such cells or organisms are known in the art (EP 0 238 023; Yelton *et al., Proc. Natl. Acad. Sci. (U.S.A.), 81*:1470-1474 (1984); Malardier *et al., Gene, 78*:147-156 (1989); Becker and Guarente, In: Abelson and Simon (eds.), *Guide to Yeast Genetics and Molecular Biology, Method Enzymol.,* Vol. 194, pp. 182-187, Academic Press, Inc., New York; Ito *et al., J. Bacteriology, 153*:163 (1983) Hinnen *et al., Proc. Natl. Acad. Sci. (U.S.A.), 75*:1920 (1978); Bennett and LaSure (eds.), *More Gene Manipulation in fungi*, Academic Press, CA (1991)). Methods to produce proteins of the present invention are also known (Kudla *et al., EMBO, 9*:1355-1364 (1990); Jarai and Buxton, *Current Genetics, 26*:2238-2244 (1994); Verdier, *Yeast, 6*:271-297 (1990; MacKenzie *et al., Journal of Gen. Microbiol., 139*:2295-2307 (1993); Hartl *et al., TIBS, 19*:20-25 (1994); Bergenron *et al., TIBS, 19*:124-128 (1994); Demolder *et al., J. Biotechnology, 32*:179-189 (1994); Craig, *Science, 260:*1902-1903 (1993); Gething and Sambrook, *Nature, 355*:33-45 (1992); Puig and Gilbert, J. *Biol. Chem., 269*:7764-7771 (1994); Wang and Tsou, *FASEB Journal, 7*:1515-1517 (1993); Robinson *et al., Bio/Technology, 1*:381-384 (1994); Enderlin and Ogrydziak, *Yeast, 10*:67-79 (1994); Fuller *et al., Proc. Natl. Acad. Sci. (U.S.A.), 86*:1434-1438 (1989); Julius *et al., Cell, 37*:1075-1089 (1984); Julius *et al., Cell 32*:839-852 (1983).

In a preferred embodiment, overexpression of a protein or fragment thereof of the present invention in a cell or organism provides in that cell or organism, relative to an untransformed cell or organism with a similar genetic background, an increased level of tocopherols.

In a preferred embodiment, overexpression of a protein or fragment thereof of the present invention in a cell or organism provides in that cell or organism, relative to an untransformed cell or organism with a similar genetic background, an increased level of α-tocopherols.

In a preferred embodiment, overexpression of a protein or fragment thereof of the present invention in a cell or organism provides in that cell or organism, relative to an untransformed cell or organism with a similar genetic background, an increased level of γ-tocopherols.

In another preferred embodiment, overexpression of a protein or fragment thereof of the present invention in a cell or organism provides in that cell or organism, relative to

an untransformed cell or organism with a similar genetic background, an increased level of α-tocotrienols.

In another preferred embodiment, overexpression of a protein or fragment thereof of the present invention in a cell or organism provides in that cell or organism, relative to an untransformed cell or organism with a similar genetic background, an increased level of γ-tocotrienols.

ANTIBODIES

One aspect of the invention concerns antibodies, single-chain antigen binding molecules, or other proteins that specifically bind to one or more of the protein or peptide molecules of the invention and their homologs, fusions or fragments. In a particularly preferred embodiment, the antibody specifically binds to a protein having the amino acid sequence set forth in SEQ ID NOs: 19-31, 33-38, 39-41, and 46-49 or a fragment thereof. In another embodiment, the antibody specifically binds to a fusion protein comprising an amino acid sequence selected from the amino acid sequence set forth in SEQ ID NOs: 19-33 and 33-38 or a fragment thereof. In another embodiment the antibody specifically binds to a fusion protein comprising an amino acid sequence selected from the amino acid sequence set forth in SEQ ID NOs: 46-49 or a fragment thereof. Antibodies of the invention may be used to quantitatively or qualitatively detect the protein or peptide molecules of the invention, or to detect post translational modifications of the proteins. As used herein, an antibody or peptide is said to "specifically bind" to a protein or peptide molecule of the invention if such binding is not competitively inhibited by the presence of non-related molecules.

Nucleic acid molecules that encode all or part of the protein of the invention can be expressed, via recombinant means, to yield protein or peptides that can in turn be used to elicit antibodies that are capable of binding the expressed protein or peptide. Such antibodies may be used in immunoassays for that protein. Such protein-encoding molecules, or their fragments may be a "fusion" molecule (*i.e.*, a part of a larger nucleic acid molecule) such that, upon expression, a fusion protein is produced. It is understood that any of the nucleic acid molecules of the invention may be expressed, via recombinant means, to yield proteins or peptides encoded by these nucleic acid molecules.

The antibodies that specifically bind proteins and protein fragments of the invention may be polyclonal or monoclonal and may comprise intact immunoglobulins, or antigen binding portions of immunoglobulins fragments (such as (F(ab'), $F(ab')_2$), or

single-chain immunoglobulins producible, for example, via recombinant means. It is understood that practitioners are familiar with the standard resource materials that describe specific conditions and procedures for the construction, manipulation and isolation of antibodies (*see*, for example, Harlow and Lane, In: *Antibodies: A Laboratory Manual,* Cold Spring Harbor Press, Cold Spring Harbor, New York (1988)).

As discussed below, such antibody molecules or their fragments may be used for diagnostic purposes. Where the antibodies are intended for diagnostic purposes, it may be desirable to derivatize them, for example with a ligand group (such as biotin) or a detectable marker group (such as a fluorescent group, a radioisotope or an enzyme).

The ability to produce antibodies that bind the protein or peptide molecules of the invention permits the identification of mimetic compounds derived from those molecules. These mimetic compounds may contain a fragment of the protein or peptide or merely a structurally similar region and nonetheless exhibits an ability to specifically bind to antibodies directed against that compound.

EXEMPLARY USES

Nucleic acid molecules and fragments thereof of the invention may be employed to obtain other nucleic acid molecules from the same species (nucleic acid molecules from corn may be utilized to obtain other nucleic acid molecules from corn). Such nucleic acid molecules include the nucleic acid molecules that encode the complete coding sequence of a protein and promoters and flanking sequences of such molecules. In addition, such nucleic acid molecules include nucleic acid molecules that encode for other isozymes or gene family members. Such molecules can be readily obtained by using the above-described nucleic acid molecules or fragments thereof to screen cDNA or genomic libraries. Methods for forming such libraries are well known in the art.

Nucleic acid molecules and fragments thereof of the invention may also be employed to obtain nucleic acid homologs. Such homologs include the nucleic acid molecules of plants and other organisms, including bacteria and fungi, including the nucleic acid molecules that encode, in whole or in part, protein homologues of other plant species or other organisms, sequences of genetic elements, such as promoters and transcriptional regulatory elements. Such molecules can be readily obtained by using the above-described nucleic acid molecules or fragments thereof to screen cDNA or genomic libraries obtained from such plant species. Methods for forming such libraries are well known in the art. Such homolog molecules may differ in their nucleotide sequences

from those found in one or more of SEQ ID NOs: 2-17, 50, and 85 and complements thereof because complete complementarity is not needed for stable hybridization. The nucleic acid molecules of the invention therefore also include molecules that, although capable of specifically hybridizing with the nucleic acid molecules may lack "complete complementarity."

Any of a variety of methods may be used to obtain one or more of the above-described nucleic acid molecules (Zamechik *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 83*:4143-4146 (1986); Goodchild *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 85*:5507-5511 (1988); Wickstrom *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 85*:1028-1032 (1988); Holt *et al.*, *Molec. Cell. Biol. 8*:963-973 (1988); Gerwirtz *et al.*, *Science 242*:1303-1306 (1988); Anfossi *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 86*:3379-3383 (1989); Becker *et al.*, *EMBO J. 8*:3685-3691 (1989)). Automated nucleic acid synthesizers may be employed for this purpose. In lieu of such synthesis, the disclosed nucleic acid molecules may be used to define a pair of primers that can be used with the polymerase chain reaction (Mullis *et al.*, *Cold Spring Harbor Symp. Quant. Biol. 51*:263-273 (1986); Erlich *et al.*, European Patent 50,424; European Patent 84,796; European Patent 258,017; European Patent 237,362; Mullis, European Patent 201,184; Mullis *et al.*, U.S. Patent 4,683,202; Erlich, U.S. Patent 4,582,788; and Saiki *et al.*, U.S. Patent 4,683,194) to amplify and obtain any desired nucleic acid molecule or fragment.

Promoter sequences and other genetic elements, including but not limited to transcriptional regulatory flanking sequences, associated with one or more of the disclosed nucleic acid sequences can also be obtained using the disclosed nucleic acid sequence provided herein. In one embodiment, such sequences are obtained by incubating nucleic acid molecules of the present invention with members of genomic libraries and recovering clones that hybridize to such nucleic acid molecules thereof. In a second embodiment, methods of "chromosome walking," or inverse PCR may be used to obtain such sequences (Frohman *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 85*:8998-9002 (1988); Ohara *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 86*:5673-5677 (1989); Pang *et al.*, *Biotechniques 22:*1046-1048 (1977); Huang *et al.*, *Methods Mol. Biol. 69:*89-96 (1997); Huang *et al.*, *Method Mol. Biol. 67:*287-294 (1997); Benkel *et al.*, *Genet. Anal. 13:*123-127 (1996); Hartl *et al.*, *Methods Mol. Biol. 58:*293-301 (1996)). The term "chromosome walking" means a process of extending a genetic map by successive hybridization steps.

The nucleic acid molecules of the invention may be used to isolate promoters of cell enhanced, cell specific, tissue enhanced, tissue specific, developmentally or environmentally regulated expression profiles. Isolation and functional analysis of the 5' flanking promoter sequences of these genes from genomic libraries, for example, using genomic screening methods and PCR techniques would result in the isolation of useful promoters and transcriptional regulatory elements. These methods are known to those of skill in the art and have been described (See, for example, Birren *et al.*, *Genome Analysis*: *Analyzing DNA*, 1, (1997), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). Promoters obtained utilizing the nucleic acid molecules of the invention could also be modified to affect their control characteristics. Examples of such modifications would include but are not limited to enhancer sequences. Such genetic elements could be used to enhance gene expression of new and existing traits for crop improvement.

Another subset of the nucleic acid molecules of the invention includes nucleic acid molecules that are markers. The markers can be used in a number of conventional ways in the field of molecular genetics. Such markers include nucleic acid molecules SEQ ID NOs: 2-17, 50, and 85, complements thereof, and fragments of either that can act as markers and other nucleic acid molecules of the present invention that can act as markers.

Genetic markers of the invention include "dominant" or "codominant" markers. "Codominant markers" reveal the presence of two or more alleles (two per diploid individual) at a locus. "Dominant markers" reveal the presence of only a single allele per locus. The presence of the dominant marker phenotype (e.g., a band of DNA) is an indication that one allele is in either the homozygous or heterozygous condition. The absence of the dominant marker phenotype (*e.g.*, absence of a DNA band) is merely evidence that "some other" undefined allele is present. In the case of populations where individuals are predominantly homozygous and loci are predominately dimorphic, dominant and codominant markers can be equally valuable. As populations become more heterozygous and multi-allelic, codominant markers often become more informative of the genotype than dominant markers. Marker molecules can be, for example, capable of detecting polymorphisms such as single nucleotide polymorphisms (SNPs).

The genomes of animals and plants naturally undergo spontaneous mutation in the course of their continuing evolution (Gusella, *Ann. Rev. Biochem. 55*:831-854

(1986)). A "polymorphism" is a variation or difference in the sequence of the gene or its flanking regions that arises in some of the members of a species. The variant sequence and the "original" sequence co-exist in the species' population. In some instances, such co-existence is in stable or quasi-stable equilibrium.

A polymorphism is thus said to be "allelic," in that, due to the existence of the polymorphism, some members of a population may have the original sequence (i.e., the original "allele") whereas other members may have the variant sequence (i.e., the variant "allele"). In the simplest case, only one variant sequence may exist and the polymorphism is thus said to be di-allelic. In other cases, the species' population may contain multiple alleles and the polymorphism is termed tri-allelic, etc. A single gene may have multiple different unrelated polymorphisms. For example, it may have a di-allelic polymorphism at one site and a multi-allelic polymorphism at another site.

The variation that defines the polymorphism may range from a single nucleotide variation to the insertion or deletion of extended regions within a gene. In some cases, the DNA sequence variations are in regions of the genome that are characterized by short tandem repeats (STRs) that include tandem di- or tri-nucleotide repeated motifs of nucleotides. Polymorphisms characterized by such tandem repeats are referred to as "variable number tandem repeat" ("VNTR") polymorphisms. VNTRs have been used in identity analysis (Weber, U.S. Patent 5,075,217; Armour *et al., FEBS Lett. 307*:113-115 (1992); Jones *et al., Eur. J. Haematol. 39*:144-147 (1987); Horn *et al.*, PCT Patent Application WO 91/14003; Jeffreys, European Patent Application 370,719; Jeffreys, U.S. Patent 5,175,082; Jeffreys *et al., Amer. J. Hum. Genet. 39*:11-24 (1986); Jeffreys *et al., Nature 316*:76-79 (1985); Gray *et al., Proc. R. Acad. Soc. Lond. 243*:241-253 (1991); Moore *et al., Genomics 10*:654-660 (1991); Jeffreys *et al., Anim. Genet. 18*:1-15 (1987); Hillel *et al., Anim. Genet. 20*:145-155 (1989); Hillel *et al., Genet. 124*:783-789 (1990)).

The detection of polymorphic sites in a sample of DNA may be facilitated through the use of nucleic acid amplification methods. Such methods specifically increase the concentration of polynucleotides that span the polymorphic site, or include that site and sequences located either distal or proximal to it. Such amplified molecules can be readily detected by gel electrophoresis or other means.

In an alternative embodiment, such polymorphisms can be detected through the use of a marker nucleic acid molecule that is physically linked to such polymorphism(s). For this purpose, marker nucleic acid molecules comprising a nucleotide sequence of a

polynucleotide located within 1 mb of the polymorphism(s) and more preferably within 100kb of the polymorphism(s) and most preferably within 10kb of the polymorphism(s) can be employed.

The identification of a polymorphism can be determined in a variety of ways. By correlating the presence or absence of it in a plant with the presence or absence of a phenotype, it is possible to predict the phenotype of that plant. If a polymorphism creates or destroys a restriction endonuclease cleavage site, or if it results in the loss or insertion of DNA (e.g., a VNTR polymorphism), it will alter the size or profile of the DNA fragments that are generated by digestion with that restriction endonuclease. As such, organisms that possess a variant sequence can be distinguished from those having the original sequence by restriction fragment analysis. Polymorphisms that can be identified in this manner are termed "restriction fragment length polymorphisms" ("RFLPs") (Glassberg, UK Patent Application 2135774; Skolnick *et al.*, *Cytogen. Cell Genet. 32*:58-67 (1982); Botstein *et al.*, *Ann. J. Hum. Genet. 32*:314-331 (1980); Fischer *et al.*, (PCT Application WO 90/13668; Uhlen, PCT Application WO 90/11369).

Polymorphisms can also be identified by Single Strand Conformation Polymorphism (SSCP) analysis (Elles, *Methods in Molecular Medicine: Molecular Diagnosis of Genetic Diseases*, Humana Press (1996)); Orita *et al.*, *Genomics 5:*874-879 (1989)). A number of protocols have been described for SSCP including, but not limited to, Lee *et al.*, *Anal. Biochem. 205:*289-293 (1992); Suzuki *et al.*, *Anal. Biochem. 192:*82-84 (1991); Lo *et al.*, *Nucleic Acids Research 20:*1005-1009 (1992); Sarkar *et al.*, *Genomics 13:*441-443 (1992). It is understood that one or more of the nucleic acids of the invention, may be utilized as markers or probes to detect polymorphisms by SSCP analysis.

Polymorphisms may also be found using a DNA fingerprinting technique called amplified fragment length polymorphism (AFLP), which is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA to profile that DNA (Vos *et al.*, *Nucleic Acids Res. 23*:4407-4414 (1995)). This method allows for the specific co-amplification of high numbers of restriction fragments, which can be visualized by PCR without knowledge of the nucleic acid sequence. It is understood that one or more of the nucleic acids of the invention may be utilized as markers or probes to detect polymorphisms by AFLP analysis or for fingerprinting RNA.

Polymorphisms may also be found using random amplified polymorphic DNA (RAPD) (Williams *et al.*, *Nucl. Acids Res. 18*:6531-6535 (1990)) and cleavable amplified

polymorphic sequences (CAPS) (Lyamichev *et al.*, *Science 260*:778-783 (1993)). It is understood that one or more of the nucleic acid molecules of the invention, may be utilized as markers or probes to detect polymorphisms by RAPD or CAPS analysis.

Single Nucleotide Polymorphisms (SNPs) generally occur at greater frequency than other polymorphic markers and are spaced with a greater uniformity throughout a genome than other reported forms of polymorphism. The greater frequency and uniformity of SNPs means that there is greater probability that such a polymorphism will be found near or in a genetic locus of interest than would be the case for other polymorphisms. SNPs are located in protein-coding regions and noncoding regions of a genome. Some of these SNPs may result in defective or variant protein expression (*e.g.*, as a result of mutations or defective splicing). Analysis (genotyping) of characterized SNPs can require only a plus/minus assay rather than a lengthy measurement, permitting easier automation.

SNPs can be characterized using any of a variety of methods. Such methods include the direct or indirect sequencing of the site, the use of restriction enzymes (Botstein *et al.*, *Am. J. Hum. Genet. 32*:314-331 (1980); Konieczny and Ausubel, *Plant J. 4*:403-410 (1993)), enzymatic and chemical mismatch assays (Myers *et al.*, *Nature 313*:495-498 (1985)), allele-specific PCR (Newton *et al.*, *Nucl. Acids Res. 17*:2503-2516 (1989); Wu *et al.*, *Proc. Natl. Acad. Sci. USA 86*:2757-2760 (1989)), ligase chain reaction (Barany, *Proc. Natl. Acad. Sci. USA 88*:189-193 (1991)), single-strand conformation polymorphism analysis (Labrune *et al.*, *Am. J. Hum. Genet. 48*: 1115-1120 (1991)), single base primer extension (Kuppuswamy *et al.*, *Proc. Natl. Acad. Sci. USA 88*:1143-1147 (1991)), Goelet US 6,004,744; Goelet 5,888,819), solid-phase ELISA-based oligonucleotide ligation assays (Nikiforov *et al.*, *Nucl. Acids Res. 22*:4167-4175 (1994), dideoxy fingerprinting (Sarkar *et al.*, *Genomics 13*:441-443 (1992)), oligonucleotide fluorescence-quenching assays (Livak *et al.*, *PCR Methods Appl. 4*:357-362 (1995a)), 5'-nuclease allele-specific hybridization TaqMan™ assay (Livak *et al.*, *Nature Genet. 9*:341-342 (1995)), template-directed dye-terminator incorporation (TDI) assay (Chen and Kwok, *Nucl. Acids Res. 25*:347-353 (1997)), allele-specific molecular beacon assay (Tyagi *et al.*, *Nature Biotech. 16*: 49-53 (1998)), PinPoint assay (Häff and Smirnov, *Genome Res. 7*: 378-388 (1997)), dCAPS analysis (Neff *et al.*, *Plant J. 14*:387-392 (1998)), pyrosequencing (Ronaghi *et al, Analytical Biochemistry 267*:65-71 (1999); Ronaghi *et al* PCT application WO 98/13523; Nyren *et al* PCT application WO 98/28440;

www.pyrosequencing.com), using mass spectrometry, *e.g.* the Masscode ™ system (Howbert *et al* PCT application, WO 99/05319; Howbert *et al* PCT application WO 97/27331; www.rapigene.com; Becker *et al* PCT application WO 98/26095; Becker *et al* PCT application; WO 98/12355; Becker *et al* PCT application WO 97/33000; Monforte *et al* US 5,965,363), invasive cleavage of oligonucleotide probes (Lyamichev *et al* *Nature Biotechnology 17*:292-296; www.twt.com), and using high density oligonucleotide arrays (Hacia *et al Nature Genetics 22*:164-167; www.affymetrix.com).

Polymorphisms may also be detected using allele-specific oligonucleotides (ASO), which, can be for example, used in combination with hybridization based technology including southern, northern, and dot blot hybridizations, reverse dot blot hybridizations and hybridizations performed on microarray and related technology.

The stringency of hybridization for polymorphism detection is highly dependent upon a variety of factors, including length of the allele-specific oligonucleotide, sequence composition, degree of complementarity (*i.e.* presence or absence of base mismatches), concentration of salts and other factors such as formamide, and temperature. These factors are important both during the hybridization itself and during subsequent washes performed to remove target polynucleotide that is not specifically hybridized. In practice, the conditions of the final, most stringent wash are most critical. In addition, the amount of target polynucleotide that is able to hybridize to the allele-specific oligonucleotide is also governed by such factors as the concentration of both the ASO and the target polynucleotide, the presence and concentration of factors that act to "tie up" water molecules, so as to effectively concentrate the reagents (*e.g.*, PEG, dextran, dextran sulfate, *etc.*), whether the nucleic acids are immobilized or in solution, and the duration of hybridization and washing steps.

Hybridizations are preferably performed below the melting temperature (T_m) of the ASO. The closer the hybridization and/or washing step is to the T_m, the higher the stringency. T_m for an oligonucleotide may be approximated, for example, according to the following formula: $T_m = 81.5 + 16.6 \times (\log10[Na+]) + 0.41 \times (\%G+C) - 675/n$; where [Na+] is the molar salt concentration of Na+ or any other suitable cation and n = number of bases in the oligonucleotide. Other formulas for approximating T_m are available and are known to those of ordinary skill in the art.

Stringency is preferably adjusted so as to allow a given ASO to differentially hybridize to a target polynucleotide of the correct allele and a target polynucleotide of

the incorrect allele. Preferably, there will be at least a two-fold differential between the signal produced by the ASO hybridizing to a target polynucleotide of the correct allele and the level of the signal produced by the ASO cross-hybridizing to a target polynucleotide of the incorrect allele (*e.g.*, an ASO specific for a mutant allele cross-hybridizing to a wild-type allele). In more preferred embodiments of the present invention, there is at least a five-fold signal differential. In highly preferred embodiments of the present invention, there is at least an order of magnitude signal differential between the ASO hybridizing to a target polynucleotide of the correct allele and the level of the signal produced by the ASO cross-hybridizing to a target polynucleotide of the incorrect allele.

While certain methods for detecting polymorphisms are described herein, other detection methodologies may be utilized. For example, additional methodologies are known and set forth, in Birren *et al.*, *Genome Analysis,* 4:135-186, *A Laboratory Manual. Mapping Genomes*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1999); Maliga *et al.*, *Methods in Plant Molecular Biology. A Laboratory Course Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1995); Paterson, *Biotechnology Intelligence Unit: Genome Mapping in Plants*, R.G. Landes Co., Georgetown, TX, and Academic Press, San Diego, CA (1996); *The Corn Handbook*, Freeling and Walbot, eds., Springer-Verlag, New York, NY (1994); *Methods in Molecular Medicine: Molecular Diagnosis of Genetic Diseases*, Elles, ed., Humana Press, Totowa, NJ (1996); Clark, ed., *Plant Molecular Biology: A Laboratory Manual*, Clark, ed., Springer-Verlag, Berlin, Germany (1997).

Factors for marker-assisted selection in a plant breeding program are: (1) the marker(s) should co-segregate or be closely linked with the desired trait; (2) an efficient means of screening large populations for the molecular marker(s) should be available; and (3) the screening technique should have high reproducibility across laboratories and preferably be economical to use and be user-friendly.

The genetic linkage of marker molecules can be established by a gene mapping model such as, without limitation, the flanking marker model reported by Lander and Botstein, *Genetics 121*:185-199 (1989) and the interval mapping, based on maximum likelihood methods described by Lander and Botstein, *Genetics 121*:185-199 (1989) and implemented in the software package MAPMAKER/QTL (Lincoln and Lander, *Mapping Genes Controlling Quantitative Traits Using MAPMAKER/QTL,* Whitehead Institute for Biomedical Research, Massachusetts, (1990). Additional software includes Qgene, Ver-

sion 2.23 (1996), Department of Plant Breeding and Biometry, 266 Emerson Hall, Cornell University, Ithaca, NY). Use of Qgene software is a particularly preferred approach.

A maximum likelihood estimate (MLE) for the presence of a marker is calculated, together with an MLE assuming no QTL effect, to avoid false positives. A $\log_{10}$ of an odds ratio (LOD) is then calculated as: LOD = $\log_{10}$ (MLE for the presence of a QTL/MLE given no linked QTL).

The LOD score essentially indicates how much more likely the data are to have arisen assuming the presence of a QTL than in its absence. The LOD threshold value for avoiding a false positive with a given confidence, say 95%, depends on the number of markers and the length of the genome. Graphs indicating LOD thresholds are set forth in Lander and Botstein, *Genetics 121*:185-199 (1989) and further described by Arús and Moreno-González , *Plant Breeding*, Hayward *et al.*, (eds.) Chapman & Hall, London, pp. 314-331 (1993).

In a preferred embodiment of the present invention the nucleic acid marker exhibits a LOD score of greater than 2.0, more preferably 2.5, even more preferably greater than 3.0 or 4.0 with the trait or phenotype of interest. In a preferred embodiment, the trait of interest is altered tocopherol levels or compositions or altered tocotrienol levels or compositions.

Additional models can be used. Many modifications and alternative approaches to interval mapping have been reported, including the use non-parametric methods (Kruglyak and Lander, *Genetics 139:*1421-1428 (1995)). Multiple regression methods or models can be also be used, in which the trait is regressed on a large number of markers (Jansen, *Biometrics in Plant Breeding*, van Oijen and Jansen (eds.), Proceedings of the Ninth Meeting of the Eucarpia Section Biometrics in Plant Breeding, The Netherlands, pp. 116-124 (1994); Weber and Wricke, *Advances in Plant Breeding,* Blackwell, Berlin, 16 (1994)). Procedures combining interval mapping with regression analysis, whereby the phenotype is regressed onto a single putative QTL at a given marker interval and at the same time onto a number of markers that serve as 'cofactors,' have been reported by Jansen and Stam, *Genetics 136*:1447-1455 (1994), and Zeng, *Genetics 136*:1457-1468 (1994). Generally, the use of cofactors reduces the bias and sampling error of the estimated QTL positions (Utz and Melchinger, *Biometrics in Plant Breeding,* van Oijen and Jansen (eds.) Proceedings of the Ninth Meeting of the Eucarpia Section Biometrics in

Plant Breeding, The Netherlands, pp. 195-204 (1994), thereby improving the precision and efficiency of QTL mapping (Zeng, *Genetics 136*:1457-1468 (1994)). These models can be extended to multi-environment experiments to analyze genotype-environment interactions (Jansen *et al., Theo. Appl. Genet. 91*:33-37 (1995)).

It is understood that one or more of the nucleic acid molecules of the invention may be used as molecular markers. It is also understood that one or more of the protein molecules of the invention may be used as molecular markers.

In a preferred embodiment, the polymorphism is present and screened for in a mapping population, *e.g.* a collection of plants capable of being used with markers such as polymorphic markers to map genetic position of traits. The choice of appropriate mapping population often depends on the type of marker systems employed (Tanksley *et al., J.P. Gustafson and R. Appels* (eds.). Plenum Press, New York, pp. 157-173 (1988)). Consideration must be given to the source of parents (adapted vs. exotic) used in the mapping population. Chromosome pairing and recombination rates can be severely disturbed (suppressed) in wide crosses (adapted x exotic) and generally yield greatly reduced linkage distances. Wide crosses will usually provide segregating populations with a relatively large number of polymorphisms when compared to progeny in a narrow cross (adapted x adapted).

An F_2 population is the first generation of selfing (self-pollinating) after the hybrid seed is produced. Usually a single F_1 plant is selfed to generate a population segregating for all the genes in Mendelian (1:2:1) pattern. Maximum genetic information is obtained from a completely classified F_2 population using a codominant marker system (Mather, Measurement of Linkage in Heredity: Methuen and Co., (1938)). In the case of dominant markers, progeny tests (*e.g.*, F_3, BCF_2) are required to identify the heterozygotes, in order to classify the population. However, this procedure is often prohibitive because of the cost and time involved in progeny testing. Progeny testing of F_2 individuals is often used in map construction where phenotypes do not consistently reflect genotype (*e.g.* disease resistance) or where trait expression is controlled by a QTL. Segregation data from progeny test populations *e.g.* F_3 or BCF_2) can be used in map construction. Marker-assisted selection can then be applied to cross progeny based on marker-trait map associations (F_2, F_3), where linkage groups have not been completely disassociated by recombination events (*i.e.*, maximum disequilibrium).

Recombinant inbred lines (RIL) (genetically related lines; usually >F_5, developed from continuously selfing F_2 lines towards homozygosity) can be used as a mapping population. Information obtained from dominant markers can be maximized by using RIL because all loci are homozygous or nearly so. Under conditions of tight linkage (*i.e.*, about <10% recombination), dominant and co-dominant markers evaluated in RIL populations provide more information per individual than either marker type in backcross populations (Reiter. *Proc. Natl. Acad. Sci. (U.S.A.) 89*:1477-1481 (1992)). However, as the distance between markers becomes larger (*i.e.*, loci become more independent), the information in RIL populations decreases dramatically when compared to codominant markers.

Backcross populations (*e.g.*, generated from a cross between a successful variety (recurrent parent) and another variety (donor parent) carrying a trait not present in the former) can be utilized as a mapping population. A series of backcrosses to the recurrent parent can be made to recover most of its desirable traits. Thus a population is created consisting of individuals nearly like the recurrent parent but each individual carries varying amounts or mosaic of genomic regions from the donor parent. Backcross populations can be useful for mapping dominant markers if all loci in the recurrent parent are homozygous and the donor and recurrent parent have contrasting polymorphic marker alleles (Reiter *et al., Proc. Natl. Acad. Sci. (U.S.A.) 89*:1477-1481 (1992)). Information obtained from backcross populations using either codominant or dominant markers is less than that obtained from F_2 populations because one, rather than two, recombinant gamete is sampled per plant. Backcross populations, however, are more informative (at low marker saturation) when compared to RILs as the distance between linked loci increases in RIL populations (*i.e.* about 0.15% recombination). Increased recombination can be beneficial for resolution of tight linkages, but may be undesirable in the construction of maps with low marker saturation.

Near-isogenic lines (NIL) (created by many backcrosses to produce a collection of individuals that is nearly identical in genetic composition except for the trait or genomic region under interrogation) can be used as a mapping population. In mapping with NILs, only a portion of the polymorphic loci is expected to map to a selected region.

Bulk segregant analysis (BSA) is a method developed for the rapid identification of linkage between markers and traits of interest (Michelmore *et al., Proc. Natl. Acad.*

Sci. U.S.A. 88:9828-9832 (1991)). In BSA, two bulked DNA samples are drawn from a segregating population originating from a single cross. These bulks contain individuals that are identical for a particular trait (resistant or susceptible to particular disease) or genomic region but arbitrary at unlinked regions (*i.e.* heterozygous). Regions unlinked to the target region will not differ between the bulked samples of many individuals in BSA.

In an aspect of the present invention, one or more of the nucleic molecules of the present invention are used to determine the level (*i.e.*, the concentration of mRNA in a sample, *etc.*) in a plant (preferably canola, corn, *Brassica campestris,* oilseed rape, rapeseed, soybean, crambe, mustard, castor bean, peanut, sesame, cottonseed, linseed, safflower, oil palm, flax or sunflower) or pattern (*i.e.*, the kinetics of expression, rate of decomposition, stability profile, *etc.*) of the expression of a protein encoded in part or whole by one or more of the nucleic acid molecule of the present invention (collectively, the "Expression Response" of a cell or tissue).

As used herein, the Expression Response manifested by a cell or tissue is said to be "altered" if it differs from the Expression Response of cells or tissues of plants not exhibiting the phenotype. To determine whether an Expression Response is altered, the Expression Response manifested by the cell or tissue of the plant exhibiting the phenotype is compared with that of a similar cell or tissue sample of a plant not exhibiting the phenotype. As will be appreciated, it is not necessary to re-determine the Expression Response of the cell or tissue sample of plants not exhibiting the phenotype each time such a comparison is made; rather, the Expression Response of a particular plant may be compared with previously obtained values of normal plants. As used herein, the phenotype of the organism is any of one or more characteristics of an organism (*e.g.* disease resistance, pest tolerance, environmental tolerance such as tolerance to abiotic stress, male sterility, quality improvement or yield *etc.*). A change in genotype or phenotype may be transient or permanent. Also as used herein, a tissue sample is any sample that comprises more than one cell. In a preferred aspect, a tissue sample comprises cells that share a common characteristic (e.g. Derived from root, seed, flower, leaf, stem or pollen etc.).

In one aspect of the present invention, an evaluation can be conducted to determine whether a particular mRNA molecule is present. One or more of the nucleic acid molecules of the present invention are utilized to detect the presence or quantity of the

mRNA species. Such molecules are then incubated with cell or tissue extracts of a plant under conditions sufficient to permit nucleic acid hybridization. The detection of double-stranded probe-mRNA hybrid molecules is indicative of the presence of the mRNA; the amount of such hybrid formed is proportional to the amount of mRNA. Thus, such probes may be used to ascertain the level and extent of the mRNA production in a plant's cells or tissues. Such nucleic acid hybridization may be conducted under quantitative conditions (thereby providing a numerical value of the amount of the mRNA present). Alternatively, the assay may be conducted as a qualitative assay that indicates either that the mRNA is present, or that its level exceeds a user set, predefined value.

A number of methods can be used to compare the expression response between two or more samples of cells or tissue. These methods include hybridization assays, such as northerns, RNAse protection assays, and *in situ* hybridization. Alternatively, the methods include PCR-type assays. In a preferred method, the expression response is compared by hybridizing nucleic acids from the two or more samples to an array of nucleic acids. The array contains a plurality of suspected sequences known or suspected of being present in the cells or tissue of the samples.

An advantage of *in situ* hybridization over more conventional techniques for the detection of nucleic acids is that it allows an investigator to determine the precise spatial population (Angerer *et al., Dev. Biol. 101*:477-484 (1984); Angerer *et al., Dev. Biol. 112*:157-166 (1985); Dixon *et al., EMBO J. 10*:1317-1324 (1991)). *In situ* hybridization may be used to measure the steady-state level of RNA accumulation (Hardin *et al., J. Mol. Biol. 202*:417-431 (1989)). A number of protocols have been devised for *in situ* hybridization, each with tissue preparation, hybridization and washing conditions (Meyerowitz, *Plant Mol. Biol. Rep. 5*:242-250 (1987); Cox and Goldberg, In: *Plant Molecular Biology: A Practical Approach,* Shaw (ed.), pp. 1-35, IRL Press, Oxford (1988); Raikhel *et al., In situ RNA hybridization in plant tissues*, In: *Plant Molecular Biology Manual*, vol. B9:1-32, Kluwer Academic Publisher, Dordrecht, Belgium (1989)).

In situ hybridization also allows for the localization of proteins within a tissue or cell (Wilkinson, *In Situ Hybridization,* Oxford University Press, Oxford (1992); Langdale, *In Situ Hybridization* In: *The Corn Handbook,* Freeling and Walbot (eds.), pp. 165-179, Springer-Verlag, New York (1994)). It is understood that one or more of the molecules of the invention, preferably one or more of the nucleic acid molecules or fragments

thereof of the invention or one or more of the antibodies of the invention may be utilized to detect the level or pattern of a protein or mRNA thereof by *in situ* hybridization.

Fluorescent *in situ* hybridization allows the localization of a particular DNA sequence along a chromosome, which is useful, among other uses, for gene mapping, following chromosomes in hybrid lines, or detecting chromosomes with translocations, transversions or deletions. *In situ* hybridization has been used to identify chromosomes in several plant species (Griffor *et al., Plant Mol. Biol. 17*:101-109 (1991); Gustafson *et al., Proc. Natl. Acad. Sci. (U.S.A.) 87*:1899-1902 (1990); Mukai and Gill, *Genome 34*:448-452 (1991); Schwarzacher and Heslop-Harrison, *Genome 34*:317-323 (1991); Wang *et al., Jpn. J. Genet. 66*:313-316 (1991); Parra and Windle, *Nature Genetics 5*:17-21 (1993)). It is understood that the nucleic acid molecules of the invention may be used as probes or markers to localize sequences along a chromosome.

Another method to localize the expression of a molecule is tissue printing. Tissue printing provides a way to screen, at the same time on the same membrane many tissue sections from different plants or different developmental stages (Yomo and Taylor, *Planta 112*:35-43 (1973); Harris and Chrispeels, *Plant Physiol.* 56:292-299 (1975); Cassab and Varner, *J. Cell. Biol. 105*:2581-2588 (1987); Spruce *et al., Phytochemistry 26:*2901-2903 (1987); Barres *et al., Neuron 5:*527-544 (1990); Reid and Pont-Lezica, *Tissue Printing: Tools for the Study of Anatomy, Histochemistry and Gene Expression,* Academic Press, New York, New York (1992); Reid *et al., Plant Physiol. 93:*160-165 (1990); Ye *et al., Plant J. 1:*175-183 (1991)).

One skilled in the art can refer to general reference texts for detailed descriptions of known techniques discussed herein or equivalent techniques. These texts include *Current Protocols in Molecular Biology* Ausubel, *et al.,* eds., John Wiley & Sons, N.Y. (1989), and supplements through September (1998), *Molecular Cloning, A Laboratory Manual,* Sambrook *et al,* 2nd Ed., Cold Spring Harbor Press, Cold Spring Harbor, New York (1989), *Genome Analysis: A Laboratory Manual 1: Analyzing DNA*, Birren *et al.,* Cold Spring Harbor Press, Cold Spring Harbor, New York (1997); *Genome Analysis: A Laboratory Manual 2: Detecting Genes*, Birren *et al.,* Cold Spring Harbor Press, Cold Spring Harbor, New York (1998); *Genome Analysis: A Laboratory Manual 3: Cloning Systems*, Birren *et al.,* Cold Spring Harbor Press, Cold Spring Harbor, New York (1999); *Genome Analysis: A Laboratory Manual 4: Mapping Genomes*, Birren *et al.,* Cold Spring Harbor Press, Cold Spring Harbor, New York (1999); *Plant Molecular Biology:*

A Laboratory Manual, Clark, Springer-Verlag, Berlin, (1997), *Methods in Plant Molecular Biology*, Maliga *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1995). These texts can, of course, also be referred to in making or using an aspect of the invention. It is understood that any of the agents of the invention can be substantially purified and/or be biologically active and/or recombinant.

Having now generally described the invention, the same will be more readily understood through reference to the following examples that are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

EXAMPLE 1

A DNA sequence of gamma-tocopherol methyltransferase from *Arabidopsis thaliana* (NCBI General Identifier Number 4106537) is used to search databases for plant sequences with homology to GMT using BLASTN (Altschul *et al.*, *Nucleic Acids Res.* *25*:3389-3402 (1997); see also www.ncbi.nlm.nih.gov/BLAST/). Results are shown in table 1, below.

TABLE 1

BLAST RESULTS FOR PLANT SEQUENCES ENCODING POLYPEPTIDES HOMOLOGOUS TO *ARABIDOPSIS* GAMMA-TOCOPHEROL METHYLTRANSFERASE

Sequences producing significant alignments:	Score (bits)	E Value
Arabidopsis thaliana (Columbia ecotype)	707	0.0
Brassica napus S8 clone	611	e-179
Brassica napus P4 clone	605	e-177
cotton GMT	459	e-133
soybeanGMT2	454	e-132
soybeanGMT1	453	e-132
soybeanGMT3	453	e-131
Marigold GMT (Tagetes erecta)	446	e-129
tomato GMT	441	e-128
cuphea GMT	440	e-127
Rice GMT	430	e-124
corn GMT	428	e-123

Sequences producing significant alignments:	Score (bits)	E Value
sorghum bicolor GMT	328	9e-94

The protein identity of these sequences compared to one another is listed in table 2.

TABLE 2

PROTEIN IDENTITY TABLE OF PLANT SEQUENCES ENCODING POLYPEPTIDES HOMOLOGOUS TO GAMMA-TOCOPHEROL METHYLTRANSFERASE

	Arabidopsis GMT (gi 4106537)	*Arabidopsis* Columbia	*Brassica* S8	*Brassica* P4	*Cuphea pulcherrima*	*Gossypium hirsutum*	*Zea mays*	*Oryza sativa*	*Sorghum bicolor*	*Tagetes erecta*
Arabidopsis GMT (gi 4106537)	100% 348/348									
Arabidopsis Columbia GMT	99% 347/348	100% 348/348								
Brassica S8 GMT	88% 309/350	88% 308/350	100% 347/347							
Brassica P4 GMT	87% 304/349	86% 303/349	96% 335/348	100% 347/347						
Cuphea pulcherrima GMT	72% 213/295	71% 212/295	68% 216/314	68% 213/313	100% 376/376					
Gossypium hirsutum GMT	67% 218/323	67% 219/323	71% 225/316	67% 231/342	71% 212/296	100% 345/345				

	Arabidopsis GMT (gi 4106537)	*Arabidopsis* Columbia	*Brassica* S8	*Brassica* P4	*Cuphea pulcherrima*	*Gossypium hirsutum*	*Zea mays*	*Oryza sativa*	*Sorghum bicolor*	*Tagetes erecta*
Zea mays GMT	63% 210/333	62% 209/333	65% 217/332	63% 211/330	71% 208/290	67% 223/331	100% 352/352			
Oryza sativa GMT	63% 212/332	63% 212/332	67% 214/319	62% 220/352	70% 204/291	65% 226/347	76% 279/364	100% 364/364		
Sorghum bicolor GMT	72% 154/212	72% 153/212	75% 159/212	73% 156/212	74% 157/212	78% 166/212	96% 208/215	91% 193/212	100% 215/215	
Tagetes erecta GMT	69% 218/312	70% 219/312	69% 214/309	68% 211/310	72% 210/291	70% 209/297	70% 216/305	71% 219/308	77% 165/212	100% 310/310
Lycopersicon esculentum GMT	68% 212/311									
Glycine max GMT1	73% 218/297									
Glycine max GMT2	70% 225/318									
Glycine max GMT3	75% 220/290									

A protein sequence of the *Synechocystis* GMT (NCBI General Identifier Number 1001725) is used in a BlastP search against predicted ORFs from other cyanobacteria at the ERGO website (www.integratedgenomics.com/IGwit/).

Two sequences with substantial homology to the *Synechocystis* GMT are found from two cyanobacteria species. These sequences are annotated as having a function of delta(24)-sterol C-methyltransferase (EC 2.1.1.41).

	E-Value	Score
Nostoc punctiforme	1e-105	375
Anabaena sp.	1e-101	361

TABLE 3

CYANOBACTERIA GMT CLUSTAL W (1.8) MULTIPLE SEQUENCE ALIGNMENT

```
Nostoc punctiforme      -------------------------
MSATLYQQIQQFYDASSGLWEQIWGEHMHHG
Anabaena sp.        ----------------------------
MSATLYQQIQQFYDASSGLWEEIWGEHMHHG
Synechocystis
MVYHVRPKHALFLAFYCYFSLLTMASATIASADLYEKIKNFYDDSSGLWEDVWGEHMHHG
                                                 ** **::*::***
******::********

Nostoc punctiforme  YYGADGTQKKDRRQAQIDLIEELLNWAGVQAAED---
LDVGCGIGGSSLYLAQKFNAKA
Anabaena sp.        YYGADGTEQKNRRQAQIDLIEELLTWAGVQTAEN---
LDVGCGIGGSSLYLAGKLNAKA
Synechocystis
YGPHGTYRIDRRQAQIDLIKELLAWAVPQNSAKPRKILDLGCGIGGSSLYLAQQHQAEV
                    ***..** : :*********:*** **  * : .   ***:************ :
*:.

Nostoc punctiforme
GITLSPVQAARATERALEANLSLRTQFQVANAQAMPFADDSFDLVWSLESGEHMPDKTK
Anabaena sp.
GITLSPVQAARATERAKEAGLSGRSQFLVANAQAMPFDDNSFDLVWSLESGEHMPDKTK
Synechocystis
MGASLSPVQVERAGERARALGLGSTCQFQVANALDLPFASDSFDWVWSLESGEHMPNKAQ
                     * :*****. ** ***   .*.   ** ****  :** .:***
**********:*::

Nostoc punctiforme FLQECYRVLKPGGKLIMVTWCHRPTD--
ESPLTADEEKHLQDIYRVYCLPYVISLPEYEA
```

```
Anabaena sp.        FLQECYRVLKPGGKLIMVTWCHRPTD--
KTPLTADEKKHLEDIYRVYCLPYVISLPEYEA
Synechocystis
FLQEAWRVLKPGGRLILATWCHRPIDPGNGPLTADERRHLQAIYDVYCLPYVVSLPDYEA
                    ****.:*******:**:.****** *  : ******.:**: **
*******:***:***

Nostoc punctiforme
IAHQLPLHNIRTADWSTAVAPFWNVVIDSAFTPQALWGLLNAGWTTIQGALSLGLMRRGY
Anabaena sp.
IARQLPLNNIRTADWSQSVAQFWNIVIDSAFTPQAIFGLLRAGWTTIQGALSLGLMRRGY
Synechocystis
IARECGFGEIKTADWSVAVAPFWDRVIESAFDPRVLWALGQAGPKIINAALCLRLMKWGY
                    **::  : :*:***** :** **: **:*** *:.::.* .** . *:.**.* **:
**

Nostoc punctiforme ERGLIRFGLLCGNK--- (SEQ ID NO: 39)
Anabaena sp.       ERGLIRFGLLCGDK--- (SEQ ID NO: 40)
Synechocystis      ERGLVRFGLLTGIKPLV (SEQ ID NO: 41)
                   ****:***** * *
```

The sequence of the Synechocystis MT1 (NCBI General Identifier Number 1653572) is used in a blast search against ESTs of other cyanobacteria at the ERGO website (www.integratedgenomics.com/IGwit/).

Three sequences with substantial homology to the Synechocystis MT1 are found from three cyanobacteria species. These sequences are all annotated as having a function of DELTA(24)- STEROL C-METHYLTRANSFERASE (EC 2.1.1.41)

BlastP	SCORE	
Anabaena sp.	1e-144	504
Synechococcus sp.	6e-98	350
Prochlorococcus marinus	2e-84	304

TABLE 4

CYANOBACTERIA MT1 CLUSTAL W (1.8) MULTIPLE SEQUENCE ALIGNMENT

```
Synechocystis    MPEYLLLPAGLISLSLAIAAGLYLLTARGYQSSDSVANAYDQWTEDGILEYYWGDHIHLG
Anabaena         -MSWLFSTLVFFLTLLTAGIALYLITARRYQSSNSVANSYDQWTEDGILEFYWGEHIHLG
```

```
Synechococcus     ---MLAGLLLLTGAAGATALLIWLQRDRRYHSSDSVAAAYDAWTDDQLLERLWGDHVHLG
Prochlorococcus   MSIFLISSLVIFLTLLFSSLILWRINTRKYISSRTVATAYDSWTQDKLLERLWGEHIHLG
                      *     :        .  ::    * * ** :** :** **:* :**  **:*:***

Synechocystis     HYGDPPVAKDFIQSKIDFVHAMAQWGGLDTLPPGTTVLDVGCGIGGSSRILAKDYGFNVT
Anabaena          HYGSPPQRKDFLVAKSDFVHEMVRWGGLDKLPPGTTLLDVGCGIGGSSRILARDYGFAVT
Synechococcus     HYGNPPGSVDFRQAKEAFVHELVRWSGLDQLPRGSRVLDVGCGIGGSARILARDYGLDVL
Prochlorococcus   FYP-LNKNIDFREAKVQFVHELVSWSGLDKLPRGSRILDVGCGIGGSSRILANYYGFNVT
                  .*       **  :*  *** :. *.*** ** *: :**********:****. **: *

Synechocystis     GITISPQQVKRATELTPPDVTAKFAVDDAMALSFPDGSFDVVWSVEAGPHMPDKAVFAKE
Anabaena          GITISPQQVQRAQELTPQELNAQFLVDDAMALSFPDNSFDVVWSIEAGPHMPDKAIFAKE
Synechococcus     GVSISPAQIRRATELTPAGLSCRFEVMDALNLQLPDRQFDAVWTVEAGPHMPDKQRFADE
Prochlorococcus   GITISPAQVKRAKELTPYECKCNFKVMDALDLKFEEGIFDGVWSVEAGAHMNNKTKFADQ
                  *::*** *::** ****   ...* * **: *.: :  ** **::***.** :*  **.:

Synechocystis     LLRVVKPGGILVVADWNQRDDRQVPLNFWEKPVMRQLLDQWSHPAFASIEGFAENLEATG
Anabaena          LMRVLKPGGIMVLADWNQRDDRQKPLNFWEKPVMQQLLDQWSHPAFSSIEGFSELLAATG
Synechococcus     LLRVLRPGGCLAAADWNRRAPKDGAMNSTERWVMRQLLNQWAHPEFASISGFRANLEASP
Prochlorococcus   MLRTLRPGGYLALADWNSRDLQKQPPSMIEKIILKQLLEQWVHPKFISINEFSSILINNK
                  ::*.::*** :. **** *  :. . .  *: :::***:** ** * **. *   *  .

Synechocystis     LVEGQVTTADWTVPTLPAWLDTIWQGIIRPQGWLQYGIRGFIKSVREVPTILLMRLAFGV
Anabaena          LVEGEVITADWTKQTLPSWLDSIWQGIVRPEGLVRFGLSGFIKSLREVPTLLLMRLAFGT
Synechococcus     HQRGLISTGDWTLATLPSWFDSIAEGLRRPWAVLGLGPKAVLQGLRETPTLLLMHWAFAT
Prochlorococcus   NSSGQVISSNWNSFTNPSWFDSIFEGMRRPNSILSLGPGAIIKSIREIPTILLMDWAFKK
                     * : :.:*.  * *:*:*:* :*: ** . :  *  ..::.:** **:***  **

Synechocystis     GLCRFGMFKAVRKNATQA----------  (SEQ ID NO: 46)
Anabaena          GLCRFGMFRALRADTVRSSAEQTSAIKVAQK  (SEQ ID NO: 47)
Synechococcus     GLMQFGVFRLSR----------  (SEQ ID NO: 48)
Prochlorococcus   GLMEFGVYKCRG----------  (SEQ ID NO: 49)
                  ** .**:::
```

EXAMPLE 2

Constructs are prepared to direct expression of the *Arabidopsis*, P4 and S8 *Brassica napus*, *Cuphea pulcherrima*, and *Gossypium hirsutum* GMT sequences in plants. The coding region of each GMT is amplified from either the appropriate EST clone or cDNA, as appropriate. Double stranded DNA sequence is obtained of all PCR products to verify that no errors are introduced by the PCR amplification.

An S8 *Brassica* GMT coding sequence is amplified from *Brassica napus* leaf cDNA as follows: PolyA$^+$ RNA is isolated from *Brassica napus* (var. Quantum) leaf tissue using an adapted biotin/streptavadin procedure based on the "mRNA Capture Kit" by Roche Molecular Biochemicals (Indianapolis, IN). Young leaf tissue is homogenized in CTAB buffer (50mM Tris-HCl pH 9, 0.8M NaCl, 0.5% CTAB, 10mM EDTA), extracted with chloroform, and pelleted. As specified by the manufacturer's instructions, polyA$^+$ RNA in the soluble fraction is hybridized to biotin-labeled oligo-dT, immobilized on streptavadin-coated PCR tubes and washed. First strand cDNA is synthesized using the "1st strand cDNA synthesis kit for RT-PCR" (Roche Molecular Biochemicals) in a 50µl volume according to the manufacturer's protocol. Following the cDNA synthesis, the soluble contents of the tube are replaced with equal volume amplification reaction mixture. Components of the mixture at final concentration consisted of: 1X Buffer 2 (Expand™ High Fidelity PCR System, Roche Molecular Biochemicals), 200µM dNTPs, 0.5 units RNAseH, 300nM each synthetic oligonucleotide primers #16879 (SEQ ID NO: 51) and #16880 (SEQ ID NO: 52) and 0.4µl Expand™ High Fidelity Polymerase (Roche Molecular Biochemicals).

A GMT gene is PCR amplified for 30 cycles using a "touchdown" cycling profile: 15 min pre-incubation at 37°C followed by a 3 min pre-incubation at 94°C, during which Expand™ polymerase is spiked into the mix. The product is then amplified for 15 cycles consisting of denaturation at 94°C for 30 sec, annealing at 65°C for 30 sec, and elongation at 72°C for 1.5 min. The annealing temperature is decreased by 1°C per cycle for each of the previous 15 cycles. An additional 15 cycles followed, consisting of 94°C for 30 sec, 50°C for 30 sec, and 72°C for 1.5 min, followed by a 7 min hold at 72°C.

The resulting PCR product is desalted using the Pharmacia "MicroSpin™ S-400 HR Column" (Pharmacia, Uppsala, Sweden) then cloned into the vector pCR2.1 using the "TOPO TA Cloning® Kit" (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. The resultant intermediate plasmid is named pMON67178 and confirmed by DNA sequencing. A pMON67178 intermediate plasmid is digested with the restriction endonucleases *Not*I and *Sse*8387I to liberate a S8 *Brassica* GMT insert, which is then gel-purified using the "QIAquick Gel Extraction Kit" (QIAGEN Inc., Valencia, CA). The vector pCGN9979 (Figure 2) is prepared by digesting with *Not*I and *Sse*8387I endonucleases. Enzymes are subsequently removed using "StrataClean Resin™" (Strat-

agene, La Jolla, CA) followed by "MicroSpin™ S-400 HR Column" treatment (Pharmacia, Uppsala, Sweden). A GMT insert is ligated into the pCGN9979 vector, resulting in the formation of the binary construct pMON67170.

An *Arabidopsis* GMT coding sequence is amplified from *Arabidopsis thaliana,* ecotype Columbia using the same methodology as described above for the S8 *Brassica* GMT with the exceptions that RNAseH is not added to the amplification reaction mixture, and the synthetic oligonucleotide primers are #16562 (SEQ ID NO: 75) and #16563 (SEQ ID NO: 76). The resulting PCR product is desalted using the Pharmacia "MicroSpin™ S-400 HR Column" (Pharmacia, Uppsala, Sweden) then cloned into the vector pCR2.1 using the "TOPO TA Cloning® Kit" (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. The resultant intermediate plasmid is named pMON67155 and confirmed by DNA sequencing. The pMON67155 intermediate plasmid is digested with the restriction endonucleases *Not*I and *Sse*8387I to liberate an *Arabidopsis thaliana* GMT insert, which is then gel-purified using the "QIAquick Gel Extraction Kit" (QIAGEN Inc., Valencia, CA). The vector pCGN9979 is prepared by digesting with *Not*I and *Sse*8387I endonucleases. Enzymes are subsequently removed using "StrataClean Resin™" (Stratagene, La Jolla, CA) followed by "MicroSpin™ S-400 HR Column" treatment (Pharmacia, Uppsala, Sweden). A GMT insert is ligated into the pCGN9979 vector, resulting in the formation of the binary construct pMON67156.

A P4 *Brassica* GMT coding sequence is amplified from *Brassica* napus leaf cDNA using the same methodology as described above for the S8 *Brassica* GMT with the exceptions that RNAseH is not added to the amplification reaction mixture, and the synthetic oligonucleotide primers are #16655 (SEQ ID NO: 53) and #16654 (SEQ ID NO: 54). A "touchdown" cycling conditions consisted of a pre-incubation for 3 min at 94°C, during which 0.4µl Expand polymerase is spiked into the mix. The product is then amplified with 15 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, and elongation at 72°C for 1.5 min. The annealing temperature is decreased by 1°C per cycle for each of the previous 15 cycles. An additional 15 cycles followed, consisting of 94°C for 30 sec, 45°C for 30 sec, and 72°C for 1.5 min, followed by a 7 min hold at 72°C.

The resulting PCR product is desalted using the Pharmacia "MicroSpin™ S-400 HR Column" (Pharmacia, Uppsala, Sweden) then cloned into the GATEWAY vector

pDONR™201 using the "PCR Cloning System with GATEWAY Technology" (Life Technologies, a Division of Invitrogen Corporation, Rockville, MD.), according to the manufacturer's instructions. The ensuing plasmid pMON68751 is confirmed by DNA sequencing.

A P4 *Brassica* GMT is then cloned from the pMON68751 donor vector into the pMON67150 destination vector, which is the GATEWAY-compatible version of the pCGN9979 Napin binary. The "*E. coli* Expression Systems with GATEWAY Technology" kit (Life Technologies, a Division of Invitrogen Corporation, Rockville, MD.) is used according to the manufacturer's instructions to create the expression clone pMON67159.

A *Cuphea pulcherrima* GMT coding sequence is amplified from the EST clone LIB3792-031-Q1-K1-F3 using the synthetic oligonucleotide primers #16658 (SEQ ID NO: 55) and #16659 (SEQ ID NO: 56). 1.0µl of EST template is used for the *Cuphea* GMT amplification reaction. Otherwise, amplification conditions and cycling parameters are identical to those of P4 *Brassica* GMT.

Using the same GATEWAY procedure as described above for the P4 *Brassica* GMT coding region, a *Cuphea* GMT PCR product is cloned into the pDONR™201 vector to create pMON68752, then subcloned into the Napin expression vector pMON67150 to create pMON67158.

A *Gossypium hirsutum* GMT coding sequence is amplified from the EST clone LIB3584-003-P1-K1-A1 using the synthetic oligonucleotide primers #16681 (SEQ ID NO: 57) and #16682 (SEQ ID NO: 58). 0.5µl of EST template is used for the *Gossypium* GMT amplification reaction. Otherwise, amplification conditions and cycling parameters are identical to those of P4 *Brassica* GMT.

Using the same GATEWAY procedure as described above for the P4 *Brassica* GMT coding region, a *Gossypium* GMT PCR product is cloned into the pDONR™201 vector to create pMON67161, then subcloned into a napin expression vector pMON67150 to create pMON67160.

The napin cassette derived from pCGN3223 (described in U.S. Patent 5,639,790) is used to drive the expression of GMT sequences in seeds. GMT sequences are cloned into the multiple cloning site of the napin cassette using either a *Not*/*Sse*8387I digest (pMON67178) or the gateway cloning system (Gibco BRL) in a binary vector suitable for plant transformation (pCGN9979).

The resulting plasmids containing the gene of interest in the plant binary transformation vector under the control of the napin promoter are labeled as follows pMON67156 (*Arabidopsis thaliana*, Columbia ecotype), pMON67170 (S8 *Brassica napus* GMT), pMON67159 (P4 *Brassica napus* GMT), pMON 67158 (*Cuphea pulcherrima* GMT), and pMON 67160 (*Gossypium hirsutum* GMT).

The plant binary constructs described above are used in *Arabidopsis thaliana* plant transformation to direct the expression of the gamma-methyltransferases in the embryo. Binary vector constructs are transformed into ABI strain *Agrobacterium* cells by the method of Holsters *et al. Mol. Gen. Genet. 163*:181-187 (1978). Transgenic *Arabidopsis thaliana* plants are obtained by *Agrobacterium*-mediated transformation as described by Valverkens *et al., Proc. Nat. Acad. Sci. 85*:5536-5540 (1988), Bent *et al., Science* 265:1856-1860 (1994), and Bechtold *et al., C.R. Acad. Sci., Life Sciences* 316:1194-1199 (1993). Transgenic plants are selected by sprinkling the transformed T_1 seeds directly onto soil and then vernalizing them at 4°C in the absence of light for 4 days. The seeds are then transferred to 21°C, 16 hours light and sprayed with a 1:200 dilution of Finale (Basta) at 7 days and 14 days after seeding. Transformed plants are grown to maturity and the T_2 seed that is produced is analyzed for tocopherol content. Figures 21a, 21b, 22a, and 22b show the tocopherol analysis from T2 seed of transgenic *Arabidopsis thaliana* plants expressing GMTs from different sources under the control of the napin seed-specific promoter. Figure 23 is a graph showing average seed α-tocopherol levels for various lines of transformed plants. In Figure 23, the plant lines shown have the following GMT sequence origins: 67156 = *Arabidopsis* GMT, 67158 = Cuphea GMT, 67159 = Brassica (P4)GMT, 67160 = Cotton GMT, and 67170 = Brassica (S8) GMT. Table 5 below gives specific tocopherol level results for various transformed and control plant lines.

TABLE 5

ng α toco/mg seed	ng β toco/mg seed	ng γ toco/mg seed	ng δ toco/mg seed	ng total toco/mg seed	Line Number	A. description	Gen.	% Alpha	Avg. Alpha %
6.64	15.28	494.91	13.16	529.99	9979-36	9979=vector control		1.3	1.3
6.07	15.69	490.82	13.66	526.23	9979-37	9979=vector control		1.2	
6.57	16.79	492.59	12.37	528.32	9979-38	9979=vector control		1.2	
7.76	17.16	513.41	15.76	554.09	9979-39	9979=vector control		1.4	

ng α toco/mg seed	ng β toco/mg seed	ng γ toco/mg seed	ng δ toco/mg seed	ng total toco/mg seed	Line Number	A. description	Gen.	% Alpha	Avg. Alpha %
8.44	15.62	508.64	15.94	548.64	9979-40	9979=vector control		1.5	
291.45	21.86	180.41	4.96	498.69	67156-8	67156=napin GMT arab	T2	58.4	75.5
275.80	20.49	141.25	3.05	440.59	67156-6	67156=napin GMT arab	T2	62.6	
289.41	21.00	138.56	3.73	452.70	67156-12	67156=napin GMT arab	T2	63.9	
312.57	22.56	128.32	2.91	466.36	67156-5	67156=napin GMT arab	T2	67.0	
302.71	20.69	113.96	2.53	439.89	67156-3	67156=napin GMT arab	T2	68.8	
329.09	24.38	118.80	3.37	475.65	67156-1	67156=napin GMT arab	T2	69.2	
352.00	21.78	128.75	3.54	506.08	67156-9	67156=napin GMT arab	T2	69.6	
304.60	19.54	110.64	2.65	437.43	67156-11	67156=napin GMT arab	T2	69.6	
337.70	24.25	109.93	2.86	474.74	67156-15	67156=napin GMT arab	T2	71.1	
359.35	20.72	39.85	0.31	420.23	67156-13	67156=napin GMT arab	T2	85.5	
367.77	22.54	35.41	0.35	426.08	67156-14	67156=napin GMT arab	T2	86.3	
373.10	22.67	27.93	0.11	423.82	67156-10	67156=napin GMT arab	T2	88.0	
383.43	23.64	24.00	0.26	431.33	67156-2	67156=napin GMT arab	T2	88.9	
385.72	22.61	10.77	0.00	419.10	67156-4	67156=napin GMT arab	T2	92.0	
412.47	27.18	13.00	0.21	452.86	67156-7	67156=napin GMT arab	T2	91.1	
296.50	23.38	163.93	7.58	491.39	67159-3	67159=brassica P4 GMT	T2	60.3	69.1
327.29	3.46	192.06	9.38	532.18	67159-13	Brassica P4 GMT	T2	61.5	
294.64	18.61	148.42	6.93	468.60	67159-2	67159=brassica P4 GMT	T2	62.9	
309.72	21.32	152.46	6.20	489.70	67159-7	67159=brassica P4 GMT	T2	63.2	
300.73	21.11	142.66	5.67	470.18	67519-1	67159=brassica P4 GMT	T2	64.0	
305.37	20.25	141.83	7.85	475.29	67159-10	67159=brassica P4 GMT	T2	64.2	
311.90	20.92	145.60	6.91	485.33	67159-5	67159=brassica P4 GMT	T2	64.3	
289.83	19.63	128.07	6.33	443.86	67159-12	67159=brassica P4 GMT	T2	65.3	
302.93	17.84	127.91	5.36	454.03	67159-6	67159=brassica P4 GMT	T2	66.7	
348.38	19.53	103.12	7.50	478.53	67159-9	67159=brassica P4 GMT	T2	72.8	
329.10	20.27	78.65	4.28	432.30	67159-15	67159=brassica P4 GMT	T2	76.1	
359.15	23.04	70.61	4.95	457.76	67159-11	67159=brassica P4 GMT	T2	78.5	
358.83	19.79	68.26	4.79	451.67	67159-14	67159=brassica P4 GMT	T2	79.4	
398.21	19.29	32.82	3.20	453.52	67159-4	67159=brassica P4 GMT	T2	87.8	
3.97	0.00	494.67	15.15	513.79	9979-81	control		0.8	0.8
3.32	0.00	501.58	18.47	523.37	9979-82	control		0.6	
4.00	0.00	492.08	15.31	511.38	9979-83	control		0.8	
4.19	0.00	541.20	18.42	563.81	9979-84	control		0.7	
5.23	0.00	541.75	20.12	567.10	9979-85	control		0.9	
251.34	10.02	216.55	6.77	484.68	67158-8	napin Cuphea GMT	T2	51.9	77.3
325.52	10.51	156.76	5.32	498.11	67158-11	napin Cuphea GMT	T2	65.4	
338.00	10.58	155.40	5.35	509.33	67158-12	napin Cuphea GMT	T2	66.4	
322.09	8.99	139.84	4.74	475.66	67158-5	napin Cuphea GMT	T2	67.7	

ng α toco/mg seed	ng β toco/mg seed	ng γ toco/mg seed	ng δ toco/mg seed	ng total toco/mg seed	Line Number	A. description	Gen.	% Alpha	Avg. Alpha %
348.47	12.70	132.54	5.14	498.85	67158-10	napin Cuphea GMT	T2	69.9	
369.43	14.85	135.94	4.49	524.71	67158-15	napin Cuphea GMT	T2	70.4	
324.99	9.08	123.23	3.95	461.25	67158-4	napin Cuphea GMT	T2	70.5	
358.91	8.49	108.56	3.76	479.72	67158-9	napin Cuphea GMT	T2	74.8	
363.29	14.16	84.19	3.45	465.09	67158-3	napin Cuphea GMT	T2	78.1	
375.18	9.78	46.59	2.39	433.94	67158-1	napin Cuphea GMT	T2	86.5	
425.61	13.14	39.87	2.71	481.32	67158-13	napin Cuphea GMT	T2	88.4	
415.44	13.75	33.16	2.01	464.35	67158-7	napin Cuphea GMT	T2	89.5	
452.35	15.65	21.65	3.46	493.10	67158-2	napin Cuphea GMT	T2	91.7	
430.11	20.33	9.67	0.00	460.11	67158-14	napin Cuphea GMT	T2	93.5	
408.68	13.89	7.13	1.22	430.92	67158-6	napin Cuphea GMT	T2	94.8	
6.18	0.00	510.97	19.47	536.62	9979-86	control		1.2	0.9
4.33	0.00	547.85	21.06	573.24	9979-87	control		0.8	
6.28	0.00	503.21	19.67	529.17	9979-88	control		1.2	
4.35	0.00	538.55	21.08	563.98	9979-89	control		0.8	
3.45	0.00	523.43	19.31	546.19	9979-90	control		0.6	
5.52	0.47	478.70	17.54	502.23	67160-7	napin cotton GMT	T2	1.1	65.1
8.11	0.00	552.24	21.34	581.69	67160-15	napin cotton GMT	T2	1.4	
324.58	7.93	177.97	7.70	518.18	67160-9	napin cotton GMT	T2	62.6	
338.02	7.43	160.27	9.11	514.82	67160-1	napin cotton GMT	T2	65.7	
345.35	9.94	159.12	7.51	521.92	67160-5	napin cotton GMT	T2	66.2	
355.54	9.65	155.73	6.95	527.87	67160-14	napin cotton GMT	T2	67.4	
371.70	14.34	142.80	6.58	535.43	67160-2	napin cotton GMT	T2	69.4	
355.35	5.96	135.17	9.11	505.59	67160-11	napin cotton GMT	T2	70.3	
360.43	7.03	136.83	7.76	512.05	67160-6	napin cotton GMT	T2	70.4	
373.32	9.65	138.68	7.74	529.39	67160-4	napin cotton GMT	T2	70.5	
374.20	10.97	89.34	4.57	479.07	67160-3	napin cotton GMT	T2	78.1	
435.98	16.16	67.09	4.81	524.03	67160-8	napin cotton GMT	T2	83.2	
446.18	13.59	44.43	3.54	507.74	67160-12	napin cotton GMT	T2	87.9	
420.34	13.54	26.74	2.51	463.12	67160-10	napin cotton GMT	T2	90.8	
465.41	15.32	21.78	2.69	505.21	67160-13	napin cotton GMT	T2	92.1	
3.98	0.00	502.78	15.54	522.30	9979-94	control		0.8	0.8
4.27	0.00	510.20	17.15	531.62	9979-93	control		0.8	
4.42	0.00	549.18	18.50	572.10	9979-91	control		0.8	
4.43	0.00	480.59	14.35	499.38	9979-95	control		0.9	
5.22	0.00	538.48	19.08	562.78	9979-92	control		0.9	
306.93	7.18	193.74	7.25	515.10	67170-3	Brassica S8 GMT	T2	59.6	77.8

ng α toco/mg seed	ng β toco/mg seed	ng γ toco/mg seed	ng δ toco/mg seed	ng total toco/mg seed	Line Number	A. description	Gen.	% Alpha	Avg. Alpha %
364.13	8.20	151.34	5.92	529.59	67170-6	Brassica S8 GMT	T2	68.8	
355.93	6.18	137.59	5.36	505.06	67170-2	Brassica S8 GMT	T2	70.5	
381.42	8.51	142.79	6.09	538.82	67170-14	Brassica S8 GMT	T2	70.8	
372.06	5.24	130.94	4.04	512.28	67170-9	Brassica S8 GMT	T2	72.6	
368.24	7.38	108.85	4.32	488.79	67170-1	Brassica S8 GMT	T2	75.3	
374.71	5.53	97.22	3.29	480.75	67170-15	Brassica S8 GMT	T2	77.9	
419.64	11.39	88.39	4.20	523.61	67170-5	Brassica S8 GMT	T2	80.1	
408.32	3.44	88.98	6.94	507.68	67170-11	Brassica S8 GMT	T2	80.4	
438.52	10.27	55.07	3.73	507.59	67170-8	Brassica S8 GMT	T2	86.4	
452.28	12.04	49.76	2.65	516.72	67170-7	Brassica S8 GMT	T2	87.5	
461.35	10.82	51.41	2.62	526.20	67170-4	Brassica S8 GMT	T2	87.7	
458.39	10.45	17.75	1.16	487.76	67170-12	Brassica S8 GMT	T2	94.0	
5.31	0.00	528.79	20.48	554.59	1	9979		1.0	1.1
5.91	0.00	543.96	21.53	571.40	2	9979		1.0	
5.26	0.00	515.35	18.45	539.07	3	9979		1.0	
6.52	0.00	509.65	19.20	535.37	4	9979		1.2	
7.70	0.00	537.19	22.97	567.87	5	9979		1.4	
5.21	0.00	511.12	19.85	536.17	6	9979		1.0	
301.07	4.48	125.80	7.99	439.34	2-8	67159=brassica P4 GMT	T3	68.5	68.1
306.33	3.22	169.37	8.75	487.68	2-3	67159=brassica P4 GMT	T3	62.8	
320.26	6.05	167.87	8.65	502.84	2-4	67159=brassica P4 GMT	T3	63.7	
329.45	7.12	169.63	9.21	515.41	2-2	67159=brassica P4 GMT	T3	63.9	
329.53	5.80	152.26	8.99	496.59	2-5	67159=brassica P4 GMT	T3	66.4	
334.46	5.82	145.10	8.16	493.54	2-6	67159=brassica P4 GMT	T3	67.8	
335.46	4.25	141.18	8.39	489.28	2-7	67159=brassica P4 GMT	T3	68.6	
344.53	8.17	145.61	9.24	507.54	2-1	67159=brassica P4 GMT	T3	67.9	
401.15	5.41	68.31	8.01	482.88	2-9	67159=brassica P4 GMT	T3	83.1	
345.21	3.07	161.54	11.71	521.53	4-2	67159=brassica P4 GMT	T3	66.2	89.2
431.50	6.46	56.16	6.72	500.83	4-9	67159=brassica P4 GMT	T3	86.2	
445.25	5.69	20.55	7.24	478.73	4-8	67159=brassica P4 GMT	T3	93.0	
445.71	5.48	20.58	6.60	478.36	4-3	67159=brassica P4 GMT	T3	93.2	
446.77	7.74	14.86	5.03	474.41	4-7	67159=brassica P4 GMT	T3	94.2	
452.65	8.96	49.76	7.52	518.89	4-4	67159=brassica P4 GMT	T3	87.2	
454.02	8.09	14.05	5.10	481.26	4-6	67159=brassica P4 GMT	T3	94.3	
467.24	9.65	11.93	4.93	493.75	4-1	67159=brassica P4 GMT	T3	94.6	
517.68	12.95	13.39	5.10	549.12	4-5	67159=brassica P4 GMT	T3	94.3	
347.03	2.66	155.38	8.28	513.35	7-5	67159=brassica P4 GMT	T3	67.6	81.9

ng α toco/mg seed	ng β toco/mg seed	ng γ toco/mg seed	ng δ toco/mg seed	ng total toco/mg seed	Line Number	A. description	Gen.	% Alpha	Avg. Alpha %
350.32	0.48	132.12	8.20	491.12	7-7	67159=brassica P4 GMT	T3	71.3	
352.48	1.50	141.14	8.26	503.37	7-2	67159=brassica P4 GMT	T3	70.0	
367.65	1.04	134.34	7.75	510.78	7-8	67159=brassica P4 GMT	T3	72.0	
372.23	0.00	125.08	7.40	504.71	7-6	67159=brassica P4 GMT	T3	73.8	
454.16	7.27	10.99	3.38	475.80	7-4	67159=brassica P4 GMT	T3	95.5	
464.63	6.08	10.50	3.10	484.31	7-9	67159=brassica P4 GMT	T3	95.9	
467.40	6.99	11.11	3.82	489.32	7-1	67159=brassica P4 GMT	T3	95.5	
474.28	8.23	11.61	4.65	498.77	7-3	67159=brassica P4 GMT	T3	95.1	
324.79	0.00	179.06	11.83	515.68	11-7	67159=brassica P4 GMT	T3	63.0	82.2
334.92	0.00	175.60	11.84	522.35	11-2	67159=brassica P4 GMT	T3	64.1	
352.84	0.00	170.23	12.16	535.22	11-5	67159=brassica P4 GMT	T3	65.9	
425.54	4.66	49.26	5.84	485.30	11-3	67159=brassica P4 GMT	T3	87.7	
427.09	5.61	61.10	6.38	500.18	11-4	67159=brassica P4 GMT	T3	85.4	
448.32	6.34	12.02	4.67	471.35	11-6	67159=brassica P4 GMT	T3	95.1	
462.49	7.21	42.46	7.43	519.59	11-1	67159=brassica P4 GMT	T3	89.0	
464.30	4.97	12.86	5.43	487.55	11-9	67159=brassica P4 GMT	T3	95.2	
469.00	4.57	16.21	5.08	494.86	11-8	67159=brassica P4 GMT	T3	94.8	
427.19	7.33	43.05	4.39	481.96	4-9	67156=napin GMT arab	T3	88.6	94.0
429.83	3.85	47.80	3.09	484.57	4-8	67156=napin GMT arab	T3	88.7	
442.62	8.97	45.02	3.71	500.32	4-4	67156=napin GMT arab	T3	88.5	
449.25	4.88	13.31	2.54	469.99	4-2	67156=napin GMT arab	T3	95.6	
454.35	6.96	2.91	2.58	466.79	4-5	67156=napin GMT arab	T3	97.3	
459.55	7.20	2.75	1.43	470.94	4-6	67156=napin GMT arab	T3	97.6	
467.64	9.17	5.77	2.51	485.09	4-3	67156=napin GMT arab	T3	96.4	
469.22	7.89	9.04	3.43	489.58	4-1	67156=napin GMT arab	T3	95.8	
476.93	6.07	3.18	2.68	488.85	4-7	67156=napin GMT arab	T3	97.6	
341.52	0.00	152.78	6.96	501.27	7-1	67156=napin GMT arab	T3	68.1	90.7
426.76	3.74	55.93	7.18	493.62	7-2	67156=napin GMT arab	T3	86.5	
427.82	2.42	36.53	3.79	470.56	7-7	67156=napin GMT arab	T3	90.9	
448.96	3.62	8.76	3.29	464.62	7-9	67156=napin GMT arab	T3	96.6	
455.79	5.26	12.41	3.45	476.91	7-6	67156=napin GMT arab	T3	95.6	
457.18	6.56	21.53	2.89	488.16	7-5	67156=napin GMT arab	T3	93.7	
461.11	6.33	8.82	3.36	479.62	7-8	67156=napin GMT arab	T3	96.1	
462.08	7.10	16.36	3.59	489.14	7-4	67156=napin GMT arab	T3	94.5	
466.01	7.72	15.40	4.54	493.68	7-3	67156=napin GMT arab	T3	94.4	
5.09	0.00	535.79	19.35	560.22	9979- 81:@.0005.	Control		0.9	
5.37	0.00	534.93	21.47	561.77	9979- 81:@.0006.	Control		1.0	

ng α toco/mg seed	ng β toco/mg seed	ng γ toco/mg seed	ng δ toco/mg seed	ng total toco/mg seed	Line Number	A. description	Gen.	% Alpha	Avg. Alpha %
327.76	22.52	156.62	9.37	516.27	67158- 2:@.0002.	napin Cuphea GMT	T3	63.5	85.2
384.99	24.97	92.36	7.82	510.14	67158- 2:@.0001.	napin Cuphea GMT	T3	75.5	
406.19	27.74	3.42	2.12	439.47	67158- 2:@.0006.	napin Cuphea GMT	T3	92.4	
424.62	22.33	34.40	6.92	488.27	67158- 2:@.0009.	napin Cuphea GMT	T3	87.0	
432.70	25.03	52.96	8.60	519.29	67158- 2:@.0004.	napin Cuphea GMT	T3	83.3	
443.67	25.50	46.41	8.22	523.80	67158- 2:@.0003.	napin Cuphea GMT	T3	84.7	
449.38	26.25	4.06	2.34	482.03	67158- 2:@.0005.	napin Cuphea GMT	T3	93.2	
449.63	25.26	2.17	1.84	478.89	67158- 2:@.0008.	napin Cuphea GMT	T3	93.9	
451.00	25.32	6.56	2.74	485.63	67158- 2:@.0007.	napin Cuphea GMT	T3	92.9	
312.62	22.03	153.68	6.73	495.05	67158- 4:@.0007.	napin Cuphea GMT	T3	63.1	75.7
326.50	23.50	131.44	6.54	487.99	67158- 4:@.0001.	napin Cuphea GMT	T3	66.9	
327.91	22.51	143.83	7.42	501.67	67158- 4:@.0005.	napin Cuphea GMT	T3	65.4	
331.65	24.40	137.74	7.20	500.98	67158- 4:@.0009.	napin Cuphea GMT	T3	66.2	
345.95	24.75	134.17	6.75	511.62	67158- 4:@.0006.	napin Cuphea GMT	T3	67.6	
355.47	24.91	120.77	6.50	507.65	67158- 4:@.0003.	napin Cuphea GMT	T3	70.0	
448.67	24.98	0.92	1.97	476.54	67158- 4:@.0004.	napin Cuphea GMT	T3	94.2	
453.62	25.23	0.98	1.59	481.42	67158- 4:@.0008.	napin Cuphea GMT	T3	94.2	
456.45	27.19	1.34	1.92	486.91	67158- 4:@.0002.	napin Cuphea GMT	T3	93.7	
6.39	0.00	498.67	24.65	529.71	9979- 81:@.0007.	Control		1.2	
6.65	0.00	520.22	19.20	546.08	9979- -81:@.0008.	Control		1.2	
325.71	19.95	154.88	8.09	508.64	67158- 9:@.0007.	napin Cuphea GMT	T3	64.0	68.4
330.27	21.90	154.36	8.08	514.61	67158- 9:@.0005.	napin Cuphea GMT	T3	64.2	
347.97	22.33	129.57	6.54	506.41	67158- 9:@.0004.	napin Cuphea GMT	T3	68.7	
351.68	22.59	122.64	6.96	503.87	67158- 9:@.0006.	napin Cuphea GMT	T3	69.8	
353.74	22.51	118.23	6.90	501.38	67158- -9:@.0001.	napin Cuphea GMT	T3	70.6	
354.17	23.30	137.47	7.50	522.44	67158- -9:@.0002.	napin Cuphea GMT	T3	67.8	
358.21	21.84	132.99	6.76	519.80	67158- 9:@.0009.	napin Cuphea GMT	T3	68.9	
362.74	22.40	114.96	6.69	506.79	67158- 9:@.0008.	napin Cuphea GMT	T3	71.6	
362.98	24.28	124.73	6.50	518.49	67158- 9:@.0003.	napin Cuphea GMT	T3	70.0	
403.35	26.19	33.39	3.08	466.02	67158- 14:@.0003.	napin Cuphea GMT	T3	86.6	90.0
416.91	26.96	34.74	3.21	481.83	67158- 14:@.0002.	napin Cuphea GMT	T3	86.5	
423.10	22.19	36.04	3.17	484.50	67158- 14:@.0008.	napin Cuphea GMT	T3	87.3	
424.87	26.52	4.48	1.62	457.49	67158- -14:@.0004.	napin Cuphea GMT	T3	92.9	
428.75	23.34	24.92	5.13	482.14	67158- 14:@.0009.	napin Cuphea GMT	T3	88.9	
433.96	30.08	5.32	2.24	471.61	67158- -14:@.0001.	napin Cuphea GMT	T3	92.0	
434.51	29.70	20.34	1.90	486.44	67158- 14:@.0005.	napin Cuphea GMT	T3	89.3	
435.86	23.44	3.27	1.75	464.33	67158-14:@.0006.	napin Cuphea GMT	T3	93.9	
440.46	23.40	10.67	2.27	476.80	67158- 14:@.0007.	napin Cuphea GMT	T3	92.4	

EXAMPLE 3

Computer programs are used to predict the chloroplast targeting peptide cleavage sites of the plant GMT proteins. The predictions of CTPs by using two programs: "Predotar" and "ChloroP" (Center for Biological Sequence Analysis, Lyngby, Denmark) are as follows

1) Program: Predotar

Sequence ID	Score	Cut Site	P-Value
Gossypium	4.56	49 * 50	3.0496E+07
Brassica	2.27	51 * 52	2.3192E+05
Cuphea	1.96	undetermined	2.7934E-01

2) chloroplast target peptide prediction results

Number of query sequences: 5

Name	Length	Score	cTP	CS-score	cTP-length
Arabidopsis	348	0.587	Y	7.834	50
Gossypium	345	0.580	Y	4.116	48
Brassica	347	0.581	Y	8.142	51
Cuphea	376	0.573	Y	1.746	64
Zea mays	352	0.560	Y	4.808	48

Based on this information GMT proteins from plant sources are engineered to remove the predicted chloroplast target peptides to allow for the expression of the mature protein in *E. coli*. In order for these proteins to be expressed in a prokaryotic expression system, an amino terminal methionine is required. This can be accomplished, for example, by the addition of a 5' ATG. A methionine is added to each of the following amino acid sequences, which are expressed in *E. coli* with detectable GMT activity (SEQ ID NOs: 33-38 each have the added methionine as the first amino acid in the sequence): Mature S8 *Brassica napus* GMT protein as expressed *in E. coli* (SEQ ID NO: 33); Mature P4 *Brassica napus* GMT protein as expressed *in E. coli* (SEQ ID NO: 34); Mature *Cuphea pulcherrima* GMT protein as expressed *in E. coli* (SEQ ID NO: 35); Mature *Gossypium hirsutum* GMT protein as expressed *in E. coli* (SEQ ID NO: 36); Mature *Tagetes erecta* (Marigold) GMT protein as expressed *in E. coli* (SEQ ID

NO:37); Mature *Zea mays* (Corn) GMT protein as expressed *in E. coli* (SEQ ID NO: 38).

Constructs are prepared to direct expression of the mature P4 and S8 *Brassica napus*, *Cuphea pulcherrima*, *Gossypium hirsutum*, *Tagetes erecta,* and *Zea mays* GMT sequences in a prokaryotic expression vector. The mature protein-coding region of each GMT with the aminoterminal methionine, as described previously, is amplified from plasmid DNA using the following species specific oligonucleotide primers in the polymerase chain reaction (PCR). Components of each 100µl PCR reaction at final concentration consisted of: 1.0µl genomic DNA or 1.0µl plasmid DNA diluted 1:20 with water, as appropriate, 1X Buffer 2 (Expand™ High Fidelity PCR System, Roche Molecular Biochemicals), 200µM dNTPs, 300nM each, synthetic oligonucleotide primers, and 0.8µl Expand™ High Fidelity Polymerase (Roche Molecular Biochemicals, Indianapolis, IN).

"Touchdown" cycling conditions consisted of a pre-incubation for 3 min at 94°C, during which the Expand polymerase is spiked into the mix. The product is then amplified with 15 cycles of denaturation at 94°C for 45 sec, annealing at 70°C for 30 sec, and elongation at 72°C for 1.5 min. The annealing temperature is decreased by 1°C per cycle for each of the previous 15 cycles. An additional 15 cycles followed, consisting of 94°C for 45 sec, 55°C for 30 sec, and 72°C for 1.5 min, followed by a 7 min hold at 72°C.

A mature S8 *Brassica* GMT coding sequence is amplified from pMON67170 using the synthetic oligonucleotide primers: #16765 (SEQ ID NO: 59) and #16654 (SEQ ID NO: 60).

A mature P4 *Brassica* GMT coding sequence is amplified from pMON67159 using the synthetic oligonucleotide primers: #16765 (SEQ ID NO: 59) and #16654 (SEQ ID NO: 60).

A mature *Cuphea pulcherrima* GMT coding sequence is amplified from pMON67158 using the synthetic oligonucleotide primers: #16763 (SEQ ID NO: 61) and #16659 (SEQ ID NO: 62).

A mature *Gossypium hirsutum* GMT coding sequence is amplified from pMON67160 using the synthetic oligonucleotide primers: #16764 (SEQ ID NO: 63) and #16682 (SEQ ID NO: 64).

A mature *Tagetes erecta* GMT coding sequence is amplified from the EST clone LIB3100-001-Q1-M1-E2 using the synthetic oligonucleotide primers: #16766 (SEQ ID NO: 65) and #16768 (SEQ ID NO: 66).

A mature *Zea mays* GMT coding region is amplified from the EST clone LIB3689-262-Q1-K1-D6 using the synthetic oligonucleotide primers: 5'GGG GAC AAG TTT GTA CAA AAA AGC AGG CTT AGA AGG AGA TAG AAC CAT GGC CTC GTC GAC GGC TCA GGC CC3' (SEQ ID NO: 73) and 5'GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC CTG CAG GCT ACG CGG CTC CAG GCT TGC GAC AG (SEQ ID NO: 74).

A GMT coding region from *Nostoc punctiforme* (ATCC 29133) is amplified from genomic DNA. Genomic DNA is isolated from 3 day cultures of the cyanobacteria according to the procedure of Chisholm (CYANONEWS, Vol. 6. No. 3 (1990)). Cultures are centrifuged and the supernatent discarded. Pellets are suspended in 400 µl TES (TES: 2.5 ml of 1 M Tris, pH 8.5; 5 ml of 5 M NaCl; 5 ml of 500 mM EDTA, bring volume to 500 ml.) To the suspended pellet, 100 µl lysozyme (50 mg/ml) is added and the suspension incubated for 15 minutes at 37°C with occasional mixing. To this, 50 µl sarkosyl (10%) is added. Protein is extracted by adding 600 µl phenol and incubating at room temperature with gentle shaking. The phases are separated by centrifugation and the aqueous phase is transferred to a new tube. RNase is added to a final concentration of 1.0 mg/ml and the solution is incubated for 15 minutes at 37°C. To this solution 100 µl NaCl (5M), 100 µl CTAB/NaCl (CTAB/NaCl: To 80 ml of water, add 4.1 g of NaCl, then 10 g CTAB, heat to 65°C to dissolve, bring volume to 100 ml), and 600 µl chloroform are added and the solution incubated 15 minutes at room temperature with gentle shaking. The phases are separated by centrifugation and the aqueous phase is transferred to a new tube. 700 µl isopropanol is added to precipitate DNA. The sample is centrifuged for 15 minutes at 14,000 rpms in a micro-centrifuge to pellet genomic DNA. The pellet is rinsed with 70% ethanol, dried briefly in a Speedvac and the genomic DNA is suspended in 100 µl TE. DNA concentration, as determined by spectrophotometry, is 79 µg/ml.

Nostoc GMT amplification reactions contained 79 ng genomic DNA, 2.5 µl 20X dNTPs 2.5 µl of each of the following primers: 5'GGG GAC AAG TTT GTA CAA AAA AGC AGG CTT AGA AGG AGA TAG AAC CAT GAG TGC AAC ACT TTA CCA GCA AAT TC 3' (SEQ ID NO: 67) and 5'GGG GAC CAC TTT GTA CAA GAA

AGC TGG GTC CTA CTA CTT ATT GCC GCA CAG TAA GC 3' (SEQ ID NO: 68), 5 µl 10X PCR buffer 2 or 3, and 0.75 µl Expand High Fidelity DNA Polymerase. PCR conditions for amplification are as follows: 1 cycle of 94°C for 2 minutes, 10 cycles of 94°C–15 seconds; 55°C–30 seconds; and 72°C– 1.5 minutes, 15 cycles of 94°C–15 seconds; 55°C–30 seconds; and 72°C– 1.5 minutes adding 5 seconds to the 72°C extension with each cycle, 1 cycle of 72°C for 7 minutes. After amplification, samples are purified using a Qiagen PCR cleanup column, suspended in 30 µl water and 10 µl are visualized on an agarose gel.

GMT and MT1 coding sequences are amplified from genomic DNA from the cyanobacterium *Anabaena* species (ATCC 27893). DNA used for PCR amplification of *Anabaena* GMT and MT1 is isolated by collecting pellets from 3 day old cyanobacteria cultures by centrifugation. The pellet is washed with 1 ml PBS to remove media. The suspension is centrifuged and the supernatent is discarded. The pellet is resuspended in 1 ml of water and boiled for 10 minutes. *Anabaena* amplification reactions contained 10 µl boiled *Anabaena* extract, 2.5 µl 20X dNTPs 2.5 µl of each primer, 5 µl 10X PCR buffer 2 or 3, and 0.75 µl Expand High Fidelity DNA Polymerase. PCR conditions for amplification are as follows: 1 cycle of 94°C for 2 minutes, 10 cycles of 94°C–15 seconds; 55°C–30 seconds; and 72°C– 1.5 minutes, 15 cycles of 94°C–15 seconds; 55°C–30 seconds; and 72°C– 1.5 minutes adding 5 seconds to the 72°C extension with each cycle, 1 cycle of 72°C for 7 minutes. After amplification, samples are purified using a Qiagen PCR cleanup column, suspended in 30 µl water and 10 µl are visualized on an agarose gel.

Anabaena species GMT coding sequence is amplified using the synthetic oligonucleotide primers: 5'GGG GAC AAG TTT GTA CAA AAA AGC AGG CTT AGA AGG AGA TAG AAC CAT GAG TGC AAC ACT TTA CCA ACA AAT TCA G 3' (SEQ ID NO: 69) and 5'GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC CTA TCA CTT ATC CCC ACA AAG CAA CC 3' (SEQ ID NO: 70).

Anabaena species MT1 coding sequence is amplified using the synthetic oligonucleotide primers: 5'GGG GAC AAG TTT GTA CAA AAA AGC AGG CTT AGA AGG AGA TAG AAC CAT GAG TTG GTT GTT TTC TAC ACT GG 3' (SEQ ID NO: 71) and 5'GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC CTA TTA CTT TTG AGC AAC CTT GAT CG3' (SEQ ID NO: 72).

The resulting PCR products are subcloned into pDONR™201 (Life Technologies, A Division of Invitrogen Corp., Rockville, MD) using the GATEWAY cloning system (Life Technologies, A Division of Invitrogen Corp., Rockville, MD) and labeled pMON67180 (mature S8 *Brassica napus* GMT), pMON68757 (mature P4 *Brassica napus* GMT), pMON68755 (mature *Cuphea pulcherrima* GMT), pMON68756 (mature *Gossypium hirsutum* GMT), pMON68758 (mature *Tagetes erecta* GMT), pMON67182 (mature *Zea mays* GMT), pMON67520 (*Nostoc punctiforme* GMT), pMON67518 (*Anabaena* species GMT), and pMON67517 (*Anabaena* species MT1). Double stranded DNA sequence is obtained to verify that no errors are introduced by the PCR amplification.

For functional testing GMT and MT1 sequences are then recombined behind the T7 promoter in the prokaryotic expression vector pET-DEST42 (Figure 1) (Life Technologies, A Division of Invitrogen Corp., Rockville, MD) using the GATEWAY cloning system (Life Technologies, A Division of Invitrogen Corp., Rockville, MD) according to the manufacturer's protocol. The resulting expression vectors are labeled pMON67181 (mature S8 *Brassica napus* GMT), pMON67172 (mature P4 *Brassica napus* GMT), pMON67173 (mature *Cuphea pulcherrima* GMT), pMON67171 (mature *Gossypium hirsutum* GMT), pMON67177 (mature *Tagetes erecta* GMT), pMON67176 (*Nostoc punctiforme* GMT), pMON67175 (*Anabaena* species GMT), pMON67174 (*Anabaena* species MT1),and pMON67183 (*Zea mays* GMT) (see also table 6).

Table 6: Bacterial expression vectors for functional testing of methyltransferases

Construct I.D.	Gene	Source of Gene	Modifications
pMON67171*	GMT	*Gossypium hirsutum*	Mature protein
pMON67172*	GMT	*Brassica napus* P4	Mature protein
pMON67173*	GMT	*Cuphea pulcherrima*	Mature protein
pMON67174	MT1	*Anabaena*	
pMON67175	GMT	*Anabaena*	
pMON67176	GMT	*Nostoc*	
pMON67177*	GMT	*Tagetes erecta*	Mature protein
pMON67181*	GMT	*Brassica napus* S8	Mature protein
pMON67183*	GMT	*Zea mays*	Mature protein

EXAMPLE 4

Bacterial expression plasmids listed in Table 6 are transformed into expression host cells (BL21 (DE3)(Stratagene, La Jolla, CA)) prior to growth and induction. A 100 mL LB-culture with the appropriate selection antibiotic (mg/mL carbenicillin) is inoculated with an overnight starter culture of cell transformants to an OD_{600} of 0.1 and grown at 25°C, 250 rpm to an OD_{600} of 0.6. The cells are then induced by adding IPTG to a final concentration of 0.4 mM and incubating for three hours at 25°C and 200 rpm. Cultures are transferred to 250 mL polypropylene centrifuge tubes, chilled on ice for five minutes, and harvested by centrifugation at 5000 *x* g for ten minutes. The cell pellet is stored at -80°C after thoroughly aspirating off the supernatant.

Methyltransferase activity is measured *in vitro* using a modification of the method described by d'Harlingue *et al.*, 1985 d'Harlingue and Camara, *J. Biol. Chem. 260(28)*:15200-3 (1985). The cell pellet is thawed on ice and resuspended in 4 mL of extraction buffer (10 mM HEPES-KOH pH 7.8, 5 mM DTT (dithiothriotol), 1 mM AEBSF (4-(2-aminoethyl) benzenesulfonyl fluoride), 0.1 μM aprotinin, 1 μg/mL leupeptin). Cells are disrupted using a French press. Each cell suspension is run through the pressure cell twice at 20,000 psi. Triton x-100 is added to a final concentration of 1% and the cell homogenate is incubated on ice for one hour before centrifugation at 5000 g for ten minutes at 4°C. The supernatant is transferred to fresh eppendorf tubes for methyltransferase activity analysis.

Enzyme assays are performed in assay buffer containing 50 mM Tris-HCl pH 7.0 (pH 8.0 for MT1), 5 mM DTT, 100 μM substrate (γ-tocopherol or γ-tocotrienol for GMT (Calbiochem-Novabiochem Corporation, San Diego, CA); 2-methylphytylplastoquinol (racemic mixture) (2-methylphytylplastoquinol and 2,3-dimethyl-5-phytylplastoquinol are synthesized as described by Soll and Schultz 1980 (Soll, J., Schultz, G., 1980, 2-methyl-6-phytylplastoquinol and 2,3-dimethyl-5-phytylplastoquinol as precursors of tocopherol synthesis in spinach chloroplasts, Phytochemistry 19:215-218) for MT1 and TMT2), 0.1 μCi ^{14}C-SAM (48 μCi/μmole, ICN Biomedicals, Aurora, Ohio), and 0.5% tween 80 (for substrate solubility) in a final volume of 1 mL. Reactions are prepared in 10 mL polypropylene culture tubes by first adding the substrate from concentrated stocks dissolved in hexane and evaporating off the hexane under nitrogen gas flow. Tween 80 is added directly to the substrate before adding the remainder of the assay buffer less the SAM. Crude cell extract is added to the assay mix

in 50 μL volumes and the timed reactions are initiated by adding SAM. Reactions are vortexed thoroughly to dissolve all of the detergent into the mix and then incubated at 30°C in the dark for 30 minutes.

The reactions are transferred to 15 mL screw-capped glass tubes with teflon-coated caps prior to quenching and phase extracting with 4 mL of 2:1 chloroform/methanol containing 1 mg/mL of butylated hydroxytoluene (BHT for stability of the end product). These are then vortexed for at least 30 seconds and centrifuged at 800 *x* g for 5 minutes to separate the layers. If necessary, 1 mL of 0.9% NaCl is added to improve the phase separation (emulsions may form because the enzyme is added as a crude extract). The organic phase (bottom layer) of each phase extraction is transferred to a fresh 15 mL glass tube and evaporated completely under nitrogen gas flow. The reaction products are then dissolved in 200 μL of ethanol containing 1% pyrogalol and vortexed for at least 30 seconds. This is filtered through a 0.2 μm filter (Whatman PTFE) into glass inserts contained within light protected LC vials for HPLC analysis.

The HPLC (HP 1100) separation is carried out using a normal phase column (Agilent Zorbax Sil, 5 μm, 4.6 x 250 mm) with 1.5 mL/minute isocratic flow of 10% methyl-t-butyl-ether in hexane over a period of 14 minutes. Samples are injected onto the column in 50 μl volumes. Quantitation of ^{14}C-labeled reaction products is performed using a flow scintillation counter (Packard 500TR). Methyltransferase activities are calculated based on a standard curve of D-α-[5-methyl-^{14}C]-tocopherol (Amersham-Pharmacia, 57 mCi/mmol).

The assay results confirm γ-tocopherol methyltransferase activity for all GMT gene candidates listed in table 6, except for the *Brassica* P4 gene (Figure 17).

The MT1 assay results (Figure 33) indicated 2-methylphytylplastoquinol methyltransferase activity with the *Anabaena* MT1 expression product. Figures 18, 19, and 20 represent HPLC chromatograms of the MT1 assay carried out with recombinant expressed *Anabaena* MT1, with recombinant *Anabaena* MT1 without 2-methylphytylplastoquinol substrate, and an assay performed with pea chloroplast extract as a positive control for the MT1 assay, respectively.

The *Anabaena*, corn, and cotton GMTs are chosen for the purpose of comparing enzymes from microbial and monocotyledon sources versus dicotyledon plant sources for methyltransferase activity with γ-tocotrienol. Assays are run in duplicate with γ-

tocopherol assays run in parallel as controls. In both cases 100 μM of substrate is used, with the substrate as the only difference in assay conditions. The monocot GMT showed comparable methyltransferase activity with γ-tocopherol and γ-tocotrienol. In contrast the bacterial and the dicot GMT are substantially less active with γ-tocotrienol. The results of this experiment are summarized in Figure 34.

EXAMPLE 5

Seed specific expression of GMT in Brassica is obtained by linking the *Arabidopsis thaliana*, ecotype Columbia gene to the napin promoter as described here. Poly A+ RNA is isolated from *Arabidopsis thaliana,* ecotype Columbia using an adapted biotin/streptavadin procedure based on a mRNA Capture Kit" (Roche Molecular Biochemicals, Indianapolis, IN). Young leaf tissue is homogenized in CTAB buffer (50mM Tris-HCl pH9, 0.8M NaCl, 0.5% CTAB, 10 mM EDTA), extracted with chloroform and pelleted. As set forth in the manufacturer's instructions, the soluble phase is hybridized to biotin-labeled oligo-dT, immobilized on streptavadin-coated PCR tubes and washed. First strand cDNA is synthesized using the "1st strand cDNA synthesis kit for RT-PCR" (Roche Molecular Biochemicals, Indianapolis, IN). cDNA synthesis is performed according to the manufacturer's protocol and followed by RNase digestion (0.5 units RNase in 48μl for 30 min.).

Arabidopsis thaliana, ecotype Columbia is amplified using primers #16562 Arab GMT Forward-*Not* 5' GCG GCC GCA CAA TGA AAG CAA CTC TAG CAG CAC CCT C 3' (SEQ ID NO: 77) and #16563 Arab GMT Reverse-*Sse* 5' CCT GCA GGT TAG AGT GGC TTC TGG CAA GTG ATG 3' (SEQ ID NO: 78) and the "Expand High Fidelity PCR System (Roche Molecular Biochemicals, Indianapolis, IN). A GMT gene is PCR-amplified for 30 cycles using a "touchdown" cycling profile: 3 min incubation at 94°C, followed by 15 cycles of 45 seconds denaturation at 94°C, 30 seconds annealing at 60°C and 2 min extensions at 72°C. Primers are designed to add a *NotI*/ Kozak site and a 3' *Sse83871* site.

The PCR product is desalted using a Pharmacia Microspin S-400 HR Column (Pharmacia, Uppsala, Sweden). The purified fragment is inserted into pCR2.1 using a TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA) resulting in the formation of pMON67155. The nucleotide sequence of the insert, *Arabidopsis thaliana,* ecotype Columbia GMT is confirmed by DNA sequencing. The GMT insert is excised from

pMON67155 by *NotI/Sse8371* digestion. Restriction enzymes are removed using StrataClean Resin (Stratagene, La Jolla, CA) and passed through a Microspin S-400 HR Column (Pharmacia, Uppsala, Sweden). The fragment is ligated into *NotI/Sse*8387I digested, identically treated pMON11307, resulting in the formation of the binary vector pMON67157 (Figure 13).

The plant binary construct described above is used in *Brassica napus* plant transformation to direct the expression of the gamma-methyltransferases in the embryo. The vector is transformed into ABI strain *Agrobacterium* cells by the method of Holsters *et al., Mol. Gen. Genet. 163*:181-187 (1978). *Brassica* plants may be obtained by *Agrobacterium*-mediated transformation as described by Radke *et al. Plant Cell Reports 11:* 499-505 (1992) and WO 00/61771. The tocopherol level and composition of the seed from transgenic plants is analyzed using the method set forth in example 6.

Results of *Brassica* transformation are shown in Figure 24, which is a graph representing the seed α-tocopherol levels for various transformants. Table 7 represents transformation data from various lines.

Table 7

ng α toco./mg seed	ng β toco./mg seed	ng γ toco./mg seed	ng δ toco./mg seed	ng total toco./mg seed	Line Number		% Alpha	Avg. % Alpha	Description
165.07	0.00	139.34	5.33	309.74	Control - Empty Vector	R1	53.3	44.1	Control
102.41	0.00	189.34	3.76	295.50	Control	R1	34.7		Control
126.90	0.00	229.27	6.64	362.81	Control	R1	35.0		Control
139.09	0.00	230.64	5.97	375.70	Control	R1	37.0		Control
137.88	0.00	173.73	4.36	315.97	Control	R1	43.6		Control
203.16	0.00	126.41	2.74	332.31	Control	R1	61.1		Control
113.75	0.00	187.68	5.86	307.29	*Arabidopsis* GMT in Canola	R1	37.0	87.1	PMON67157-10
197.02	0.00	137.48	4.50	338.99	*Arabidopsis* GMT in Canola	R1	58.1		PMON67157-9
201.11	0.00	134.65	6.52	342.28	*Arabidopsis* GMT in Canola	R1	58.8		PMON67157-5
212.78	0.00	92.97	3.36	309.11	*Arabidopsis* GMT in Canola	R1	68.8		PMON67157-4
240.49	0.00	53.44	1.77	295.70	*Arabidopsis* GMT in Canola	R1	81.3		PMON67157-6
231.63	0.00	49.46	0.00	281.09	*Arabidopsis* GMT in Canola	R1	82.4		PMON67157-25
234.90	0.00	45.91	1.03	281.84	*Arabidopsis* GMT in Canola	R1	83.3		PMON67157-20
334.07	0.00	57.69	1.65	393.41	*Arabidopsis* GMT in Canola	R1	84.9		PMON67157-27
345.00	0.00	36.75	2.23	383.99	*Arabidopsis* GMT in Canola	R1	89.8		PMON67157-21
286.02	0.00	1.04	1.61	288.67	*Arabidopsis* GMT in Canola	R1	99.1		PMON67157-2
387.23	0.00	0.16	1.64	389.03	*Arabidopsis* GMT in Canola	R1	99.5		PMON67157-3
322.59	0.00	0.68	0.66	323.93	*Arabidopsis* GMT in Canola	R1	99.6		PMON67157-8

ng α toco./mg seed	ng β toco./mg seed	ng γ toco./mg seed	ng δ toco./mg seed	ng total toco./mg seed	Line Number		% Alpha	Avg. % Alpha	Description
331.27	0.00	0.46	0.61	332.34	*Arabidopsis* GMT in Canola	R1	99.7		PMON67157-1
322.34	0.00	0.00	0.62	322.97	*Arabidopsis* GMT in Canola	R1	99.8		PMON67157-24
316.73	0.00	0.51	0.00	317.24	*Arabidopsis* GMT in Canola	R1	99.8		PMON67157-13
357.05	0.00	0.24	0.00	357.29	*Arabidopsis* GMT in Canola	R1	99.9		PMON67157-17
310.97	0.00	0.17	0.00	311.13	*Arabidopsis* GMT in Canola	R1	99.9		PMON67157-22
324.07	0.00	0.00	0.00	324.07	*Arabidopsis* GMT in Canola	R1	100.0		PMON67157-23
367.84	0.00	0.00	0.00	367.84	*Arabidopsis* GMT in Canola	R1	100.0		PMON67157-28
438.54	0.00	0.00	0.00	438.54	*Arabidopsis* GMT in Canola	R1	100.0		PMON67157-30

EXAMPLE 6

Seed specific expression of GMT in soy is obtained by linking the *Arabidopsis thaliana*, ecotype Columbia GMT gene with different types of seed specific promoters as described here. Total RNA is isolated from *Arabidopsis* leaf tissue (ecotype Columbia) using the Qiagen "RNeasy plant mini kit" (Qiagen Inc., Valencia, CA). First strand cDNA synthesized using the "1st strand cDNA synthesis kit for RT-PCR" from Boehringer Mannheim. RNA isolation and cDNA synthesis is performed according to the manufacturer protocols.

The *Arabidopsis* GMT is amplified using primers "GMT-ara 5' CAT GCC ATG GGA ATG AAA GCA ACT CTA GCA G" (SEQ ID NO: 75) and "GMT-ara 3' GTC AGA ATT CTT ATT AGA GTG GCT TCT GGC AAG" (SEQ ID NO: 76) and the Boehringer Mannheim "Expand™ High Fidelity PCR System". The GMT gene is PCR-amplified by 30 cycles under the following conditions: 5 min incubation at 95°C, followed by 30 cycles of 1 min at 95°C, 1 min annealing at 58°C and 2 min extension at 72°C. These reactions are followed by 5 min incubation at 72°C. The primers are designed to add a methionine and a glycin to the N-terminus of the GMT protein.

The PCR products are EcoRI and NcoI digested and gel purified using the Qiagen "Qiaquick Gel Extraction Kit" (Qiagen Inc., Valencia, CA). Purified fragments are ligated into EcoRI / NocI digested and gel purified pET30 (Novagen, Madison, WI) and pSE280 (Invitrogen, Carlsbad, CA) resulting in the formation of pMON26592 (Figure 3) and pMON26593 (Figure 4), respectively. Subsequently the *Arabidopsis* GMT sequence is confirmed. During the sequencing procedure it is found that the cloned sequence from the Columbia ecotype exhibited two nucleotide changes compared to the

Arabidopsis thaliana GMT sequence published in WO 99/04622 (position 345, change from C to T; position 523, substitution from T to G). While the first substitution is a silent mutation, the second nucleotide change resulted in an amino acid change from serine to alanine.

For generation of a GMT plant transformation vector under p7S promoter control, a GMT is excised as a *Bgl*II / *Eco*RI fragment from pMON26592, gel purified, and cloned into a *Bgl*II / *Eco*RI digested and gel purified vector containing a p7S expression cassette resulting in the formation of the shuttle vector pMON36500 (Figure 6). The p7S::GMT_{At} expression cassette is excised from pMON36500 by *Pst*I digest, the ends are filled in by T4 DNA polymerase treatment, gel purified, and cloned into *Sma*I digested, alkaline phosphatase treated and gel purified pMON38207R, resulting in the formation of the binary vector pMON36503.

An *Nco*I / *Eco*RI digested, gel purified GMT excised from pMON26592 is ligated into an *Nco*I / *Eco*RI digested vector harboring a pARC5-1 expression cassette, resulting in the formation of the shuttle vector pMON36502. The pARC5-1::GMTAt expression cassette is excised from pMON35502 by *Not*I digest, blunt ends are generated by treatment with Klenow fragment, the fragment is gel purified, and ligated into *Sma* I digested, alkaline phosphatase treated and gel purified pMON38207R. The resulting binary vector is designated pMON36505.

An arcelin 5 promoter harbors 6 ATG start codons at the 5' sequence located in different reading frames (Goosens *et al., Plant Physiol.* (1999), 120(4), 1095-1104, Goosens *et al., FEBS Lett.* (1999), 456(1), 160-164.). To decrease the risk of interference of these start codons during gene expression, 4 of these putative translational start sites are deleted. Deletion of 4 ATG codons is achieved by PCR, using primers Parc5' (5'-CCA CGT GAG CTC CTT CCT CTT CCC-3') (SEQ ID NO: 79) and Parc3' (5'-GTG CCA TGG CAG ATC TGA TGA TGG ATT GAT GGA-3') (SEQ ID NO: 80). Primer Parc3' is designed to hybridize to the Arcelin 5 promoter sequence at the translational start site and delete 4 of the 6 ATG codons. PCR is performed using pMON55524 (Figure 5) as template DNA and the Boehringer Mannheim PCR Core Kit in 30 PCR cycles under the following conditions: 5 min incubation at 95°C, followed by 30 cycles of 1 min at 95°C, 1 min annealing at 60°C and 40 second extension at 72°C. These reactions are followed by 5 min incubation at 72°C. The resulting approximately 360 bp PCR product is digested with *Sal*I and *Nco*I, gel purified and cloned into

SalI/*Nco*I digested and gel purified pMON55524, resulting in the formation of pMON36501 (Figure 7). A DNA sequence of the cloned PCR product is confirmed by DNA sequencing. A GMT expression cassette using the modified promoter is assembled by ligating the backbone of *Sma*I / *Nco*I digested and gel purified pMON36501 with a GMTAt::Arcelin 5 3' terminator fusion obtained from *Sma*I / *Nco*I digested gel purified pMON36502 (Figure 8). The resulting shuttle vector is designated pMON36504 (Figure 10). A binary vector (pMON36506) harboring a GMT expression cassette under the control of the modified arcelin 5 promoter is generated by cloning the *Not*I digested, Klenow fragment treated (for blunt end generation), gel purified GMT expression cassette into gel purified *Sma*I digested alkaline phosphatase treated, and gel purified pMON38207R vector backbone (5'-GAG TGA TGG TTA ATG CAT GAA TGC ATG ATC AGA TCT GCC ATG GTC CGT CCT-3' (SEQ ID NO: 81)(original DNA sequence at the translational start site of the Arcelin 5 promoter -- pARC5-1)(5'-GAG TGA TGG TTA ATC CAT CAA TCC ATC ATC AGA TCT GCC ATG GTC CGT CCT-3') (SEQ ID NO: 82) (DNA sequence at the translational start site of the mutated Arcelin 5 promoter -- pARC5-1M))

GMT expression vectors pMON36503 (Figure 9), pMON36505 (Figure 11) and pMON36506 (Figure 12) are transformed into the soybean line A3244 using *Agrobacterium* mediated transformation. See, for example the methods described by Fraley *et al., Bio/Technology 3*:629-635 (1985) and Rogers *et al., Methods Enzymol. 153*: 253-277 (1987). Ten bulked seeds from the R_1 generation are ground and the resulting soy meal is used for tocopherol analysis. Twenty five to forty mg of the soy meal is weighed into a 2 mL micro tube, and 500 µl 1% pyrogallol (Sigma Chemicals, St. Louis, MO) in ethanol containing 5 µg/mL tocol, is added to the tube. The sample is shaken twice for 45 seconds in a FastPrep (Bio101/Savant) using speed 6.5. The extract is then filtered (Gelman PTFE acrodisc 0.2 µm, 13 mm syringe filters, Pall Gelman Laboratory Inc, Ann Arbor, MI) into an autosampler tube. HPLC is performed on a Zorbax silica HPLC column, 4.6 mm x 250 mm (5 µm) with a fluorescent detection using a Hewlett Packard HPLC (Agilent Technologies). Sample excitation is performed at 290 nm, and emission is monitored at 336 nm. Tocopherols are separated with a hexane methyl-t-butyl ether gradient using an injection volume of 20 µl, a flow rate of 1.5 ml/min, and a run time of 12 min (40°C). Tocopherol concentration and composition is calculated based on standard curves for α, β, γ and δ-tocopherol using Chemstation

software (Agilent Technologies, Palo Alto, CA). As shown in Figures 14-16, several lines from each construct completely or substantially converted δ and γ-tocopherol, leaving α and β-tocopherol as the only detectable tocopherol isomers.

EXAMPLE 7

Canola, *Brassica napus,* or soybean plants are transformed with a variety of DNA constructs using *Agrobacterium* mediated transformation. Two sets of DNA constructs are produced. The first set of constructs are "single gene constructs". Each of the following genes is inserted into a separate plant DNA construct under the control of a seed specific promoter such as the arcelin 5, 7S α or napin promoter (Kridl *et al., Seed Sci. Res.* 1:209:219 (1991) (Keegstra, *Cell 56(2)*:247-53 (1989); Nawrath, *et al., Proc. Natl. Acad. Sci. U.S.A. 91*:12760-12764 (1994)): a bifunctional prephenate dehydrogenase such as the *E. herbicola* or the *E. coli tyrA* gene (Xia *et al., J. Gen. Microbiol. 138*:1309-1316 (1992)), a phytylprenyltransferase such as the slr1736 (in Cyanobase (www.kazusa.or.jp/cyanobase)) or the ATPT2 gene (Smith *et al., Plant J. 11: 8*3-92 (1997)), a 1-deoxyxylulose 5-phosphate synthase such as the *E. coli* dxs gene (Lois *et al., Proc. Natl. Acad. Sci. U.S.A. 95 (5):*2105-2110 (1998)), a 1-deoxyxylulose 5-phosphate reductoisomerase (dxr) gene (Takahashi *et al. Proc. Natl. Acad. Sci. U.S.A. 95 (17)*, 9879-9884 (1998)), a p-hydroxyphenylpyruvate dioxygenase, such as the *Arabidopsis thaliana* HPPD gene (Norris *et al., Plant Physiol. 117*:1317-1323 (1998)), a geranylgeranylpyrophosphate synthase gene such as the *Arabidopsis thaliana* GGPPS gene (Bartley and Scolnik, *Plant Physiol. 104*:1469-1470 (1994)), a transporter such as the AANT1 gene (Saint Guily, *et al., Plant Physiol., 100(2)*:1069-1071 (1992)), a GMT gene, an MT1 gene, and a tocopherol cyclase such as the slr1737 gene (in Cyanobase (www.kazusa.or.jp/cyanobase) or its *Arabidopsis* ortholog (PIR_T04448)), a isopentenylpyrophosphate isomerase gene (IDI), and an antisense construct for homogentisic acid dioxygenase (Sato *et al., J. DNA Res. 7 (1)*:31-63 (2000))). The products of the genes are targeted to the plastid by natural plastid target peptides present in the trans gene, or by an encoded plastid target peptide such as CTP1. Each construct is transformed into at least one canola, *Brassica napus* and soybean plant. Plants expressing each of these genes are selected to participate in additional crosses. Crosses are carried out for each species to generate transgenic plants having one or more of the following combination of introduced genes: *tyrA, slr1736, ATPT2, dxs, dxr, GGPPS, HPPD,*

GMT, MT1, AANT1, slr 1737, IDI, and an antisense construct for homogentisic acid dioxygenase.

The tocopherol composition and level in each plant generated by the crosses (including all intermediate crosses) is also analyzed. Progeny of the transformants from these constructs will be crossed with each other to stack the additional genes to reach the desired level of tocopherol.

A second set of DNA constructs is generated and referred to as the "multiple gene constructs." The multiple gene constructs contain multiple genes each under the control of a seed specific promoter such as the arcelin 5, 7S α or napin promoter (Kridl *et al., Seed Sci. Res.* 1:209:219 (1991) (Keegstra, *Cell 56(2)*:247-53 (1989); Nawrath, *et al., Proc. Natl. Acad. Sci. U.S.A. 91*:12760-12764 (1994)) and the gene products of each of the genes are targeted to the plastid by an encoded plastid target peptide. The multiple gene construct can have two or more of the following genes: *tyrA, slr1736,* or *ATPT2, dxs, dxr, GGPPS, HPPD, GMT, MT1, AANT1, slr 1737,* or its plant ortholog, *IDI,* and an antisense construct for homogentisic acid dioxygenase.

Each construct is then transformed into at least one canola, *Brassica napus* or soybean plant. The tocopherol composition and level in each plant is also analyzed using the method set forth in example 6. Progeny of the transformants from these constructs are crossed with each other to stack the additional genes to reach the desired level of tocopherol.

EXAMPLE 8

Expression of the *Anabaena* MT1 coding sequence in *Arabidopsis* is carried out. The *Anabaena* putative-MT1 coding sequence is amplified from genomic DNA derived from 3-day old *Anabaena sp.* (ATCC 27893) cultures. To isolate DNA, cultures are spun and the pellet washed with 1 ml PBS to remove media. Subsequently, the suspension is centrifuged and the supernatant is discarded. The resulting pellet is resuspended in 1 ml of water and is boiled for 10 minutes. *Anabaena* DNA amplification reactions contain 10 μL boiled *Anabaena* extract, the Expand™ High Fidelity PCR System and the oligonucleotide primers: 5'GGG GAC AAG TTT GTA CAA AAA AGC AGG CTT AGA AGG AGA TAG AAC CAT GAG TTG GTT GTT TTC TAC ACT GG 3' (SEQ ID NO: 83) and 5'GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC CTA TTA CTT TTG AGC AAC CTT GAT CG3' (SEQ ID NO: 84). The reaction mix is pre-

incubated for 5 minutes at 95°C, during which time the polymerase is spiked in. The product is then amplified for 15 cycles of 94°C for 30 sec, 60°C for 30 sec, and 72°C for 1.5 minutes each. During the cycling, the annealing temperature is decreased by 1°C per cycle for each of the 15 cycles. An additional 15 cycles follow, consisting of 94°C for 30 seconds, 45°C for 30 seconds, and 72°C for 1.5 minute, each followed by a 7 minute hold at 72°C.

After amplification, PCR products are purified using a Qiagen PCR cleanup column (Qiagen Company, Valencia, California) and subcloned into pDONR™201 using the GATEWAY cloning system (Life Technologies, Rockville, MD) to generate pMON67517. Sequences are confirmed by DNA sequencing using standard methodologies and then cloned into the napin cassette derived from pCGN3223 (Kridl *et al.*, *Seed Sci. Res. 1*:209-219 (1991)) in a GATEWAY compatible binary destination vector containing the BAR selectable marker under the control of the 35S promoter. The MT1 gene is cloned in as a translational fusion with the encoded plastid target peptide CTP1 (WO 00/61771) to target this protein to the plastid from pMON16600. The resultant expression vector (pMON67211) is electroporated into ABI strain *Agrobacterium* cells and grown under standard conditions (McBride *et al.*, *Proc. Natl. Acad. Sci. USA 91*:7301-7305 (1994)) and vector fidelity is reconfirmed by restriction analysis. Transformation of pMON67211 into wild-type *Arabidopsis*, accession Columbia, as well as three high δ-tocopherol mutant lines (*hdt2, hdt10, hdt16*) is accomplished using the dipping method (Clough and Bent, *Plant J. 16(6)*:735-43 (1998)) and T_0 plants are grown in a growth chamber under 16h light, 19°C. T_1 seeds are sprinkled directly onto soil, vernalized at 4°C in the absence of light for 4 days, then transferred to 21°C, 16 hours light. Transgenic plants are selected by spraying with a 1:200 dilution of Finale (AgrEvo Environmental Health, Montvale, NJ) at 7 days and 14 days after seeding. Transformed plants are grown to maturity and the T_2 seed is analyzed for tocopherol content using normal phase HPLC (Savidge, B. *et al., Plant Physiology 129*:321-332 (2002)).

Two lines of pMON67211 in the hdt2 mutant line (67211-6 and 67211-12) are taken forward to the next generation for examination of phenotype in T_3 seed. In doing so, T_2 seeds are sprinkled directly onto soil, vernalized at 4°C in the absence of light for 4 days, then transferred to 21°C, 16 hours light. Transgenic plants are selected by spraying with a 1:200 dilution of Finale (AgrEvo Environmental Health, Montvale, NJ) at 7

days and 14 days after seeding. Transformed plants are grown to maturity (9 plants from line 6, 9 plants from line 12, and 4 *hdt2* mutant controls in one flat) and the T_3 seed is analyzed for tocopherol content using normal phase HPLC.

Figure 25 shows the percent of seed δ-tocopherol in *Arabidopsis* T2 seed from lines expressing MT1 under the control of the napin promoter.

Table 8 below represents various data resulting from the above transformants.

Table 8

Alpha strategy R_2 *Arabidopsis* seed:CTP-MT1										
HPLC sequence and data folder SR022602										
Sample Name	Sample wt. (mg)	Ng α toco./mg seed	ng γ toco./mg seed	ng δ toco./mg seed	ng total toco./mg seed	Serial Number	Pedigree	Gen	% Delta	Avg. Delta %
77	14	3.42	459.72	20.17	483.31	9979-AT00002-54:@.0008.	9979	For 67211s	4.2	4.1
78	15	2.59	461.87	19.26	483.73	9979-AT00002-54:@.0009.	9979	For 67211s	4.0	
89	13	4.78	459.21	16.34	480.33	AT_G193:@.	PMON67211	T2	3.4	3.6
90	14	5.50	475.59	17.27	498.35	AT_G194:@.	PMON67211	T2	3.5	
86	13	5.64	476.70	18.13	500.46	AT_G190:@.	PMON67211	T2	3.6	
82	13	6.19	476.84	18.10	501.13	AT_G186:@.	PMON67211	T2	3.6	
88	14	7.13	477.51	19.27	503.91	AT_G192:@.	PMON67211	T2	3.8	
95	13	6.45	478.90	18.78	504.13	AT_G199:@.	PMON67211	T2	3.7	
85	11	5.67	480.31	19.62	505.60	AT_G189:@.	PMON67211	T2	3.9	
96	13	10.08	480.68	18.69	509.45	AT_G200:@.	PMON67211	T2	3.7	
84	13	6.34	487.23	18.47	512.04	AT_G188:@.	PMON67211	T2	3.6	
91	12	7.18	487.68	19.42	514.28	AT_G195:@.	PMON67211	T2	3.8	
87	14	4.45	492.16	19.92	516.52	AT_G191:@.	PMON67211	T2	3.9	
93	13	7.07	492.17	18.19	517.43	AT_G197:@.	PMON67211	T2	3.5	
92	13	7.12	493.27	19.77	520.15	AT_G196:@.	PMON67211	T2	3.8	
94	13	8.28	494.79	18.04	521.11	AT_G198:@.	PMON67211	T2	3.5	
80	13	8.70	498.94	18.71	526.36	AT_G184:@.	PMON67211	T2	3.6	
83	14	6.49	502.75	18.16	527.40	AT_G187:@.	PMON67211	T2	3.4	
81	12	6.75	505.87	18.84	531.45	AT_G185:@.	PMON67211	T2	3.5	
9	12	3.66	277.61	265.61	546.88	hdt2:0001.		M5	48.6	48.1
10	10	5.62	268.82	239.24	513.69	hdt2:0002.		M5	46.6	
11	13	4.80	266.70	250.79	522.29	hdt2:0003.		M5	48.0	
12	12	6.34	281.87	271.70	559.90	hdt2:0004.		M5	48.5	
13	12	4.75	277.59	266.87	549.21	hdt2:0005.		M5	48.6	

Alpha strategy R_2 *Arabidopsis* seed:CTP-MT1										
HPLC sequence and data folder SR022602										
Sample Name	Sample wt. (mg)	Ng α toco./mg seed	ng γ toco./mg seed	ng δ toco./mg seed	ng total toco./mg seed	Serial Number	Pedigree	Gen	% Delta	Avg. Delta %
18	13	4.38	410.93	146.44	561.74	67211-HDT2:0005.		T2	26.1	18.9
20	12	5.53	421.63	133.57	560.73	67211-HDT2:0007.		T2	23.8	
22	11	4.39	413.42	116.94	534.75	67211-HDT2:0009.		T2	21.9	
17	12	5.31	425.83	114.16	545.30	67211-HDT2:0004.		T2	20.9	
15	12	4.97	402.64	105.62	513.23	67211-HDT2:0002.		T2	20.6	
27	13	4.74	434.37	112.96	552.07	67211-HDT2:0014.		T2	20.5	
16	13	5.98	416.73	108.13	530.84	67211-HDT2:0003.		T2	20.4	
14	12	7.07	431.05	107.70	545.81	67211-HDT2:0001.		T2	19.7	
23	10	4.74	436.59	106.91	548.24	67211-HDT2:0010.		T2	19.5	
26	12	6.89	424.31	104.39	535.59	67211-HDT2:0013.		T2	19.5	
21	11	4.91	441.50	104.57	550.98	67211-HDT2:0008.		T2	19.0	
28	12	4.40	493.29	87.63	585.32	67211-HDT2:0015.		T2	15.0	
24	13	4.20	452.86	74.83	531.89	67211-HDT2:0011.		T2	14.1	
25	13	5.20	510.41	72.70	588.31	67211-HDT2:0012.		T2	12.4	
19	11	5.58	545.61	67.86	619.05	67211-HDT2:0006.		T2	11.0	
3	12.5	3.36	262.76	180.18	446.30	hdt16:@.0007.	Control	M5	40.4	38.2
2	9.6	2.54	305.52	178.20	486.25	hdt16:@.0005.	Control	M5	36.6	
1	11.9	3.36	290.12	177.76	471.24	hdt16:@.0003.	Control	M5	37.7	
11	10.1	2.02	255.50	169.29	426.81	AT_G58:@.	PMON67211	T2	39.7	15.3
12	12.4	5.28	352.67	100.76	458.71	AT_G59:@.	PMON67211	T2	22.0	
24	12.5	3.60	392.97	78.20	474.77	AT_G71:@.	PMON67211	T2	16.5	
14	12	3.90	380.29	72.98	457.18	AT_G61:@.	PMON67211	T2	16.0	
22	12.6	2.06	370.66	68.50	441.22	AT_G69:@.	PMON67211	T2	15.5	
18	12.2	3.52	379.38	70.29	453.19	AT_G65:@.	PMON67211	T2	15.5	
15	13	5.67	386.12	71.61	463.39	AT_G62:@.	PMON67211	T2	15.5	
21	11.3	3.86	405.98	74.54	484.39	AT_G68:@.	PMON67211	T2	15.4	
25	12.6	6.42	408.38	74.56	489.36	AT_G72:@.	PMON67211	T2	15.2	
19	12.5	3.95	412.64	72.24	488.84	AT_G66:@.	PMON67211	T2	14.8	
20	12.7	2.99	431.01	65.65	499.66	AT_G67:@.	PMON67211	T2	13.1	
17	12.3	5.77	423.19	48.73	477.70	AT_G64:@.	PMON67211	T2	10.2	
23	11.3	2.35	408.24	45.41	456.00	AT_G70:@.	PMON67211	T2	10.0	
10	11.9	7.81	443.06	43.58	494.45	AT_G57:@.	PMON67211	T2	8.8	
13	12.6	3.64	421.06	38.53	463.23	AT_G60:@.	PMON67211	T2	8.3	
16	12.9	3.76	430.69	37.10	471.56	AT_G63:@.	PMON67211	T2	7.9	
33	13.2	4.32	356.41	71.85	432.59	hdt10:@.0001.	Control	M6	16.6	9.6
34	13.1	5.73	469.11	12.79	487.62	hdt10:@.0002.	Control	M6	2.6	

Alpha strategy R_2 *Arabidopsis* seed:CTP-MT1										
HPLC sequence and data folder SR022602										
Sample Name	Sample wt. (mg)	Ng α toco./mg seed	ng γ toco./mg seed	ng δ toco./mg seed	ng total toco./mg seed	Serial Number	Pedigree	Gen	% Delta	Avg. Delta %
56	13.3	4.77	361.67	63.37	429.82	AT_G48:@.	PMON67211	T2	14.7	4.7
61	8.1	2.70	351.84	50.96	405.50	AT_G54:@.	PMON67211	T2	12.6	
54	12.2	5.66	432.55	41.60	479.81	AT_G46:@.	PMON67211	T2	8.7	
59	13.9	5.18	416.88	38.34	460.40	AT_G52:@.	PMON67211	T2	8.3	
51	13	3.99	430.18	22.41	456.58	AT_G43:@.	PMON67211	T2	4.9	
58	12.2	4.88	463.37	21.72	489.97	AT_G51:@.	PMON67211	T2	4.4	
52	13.4	5.34	442.72	18.24	466.31	AT_G44:@.	PMON67211	T2	3.9	
64	12.6	5.50	477.62	10.72	493.84	AT_G117:@.	PMON67211	T2	2.2	
57	12.7	6.27	467.48	9.12	482.88	AT_G50:@.	PMON67211	T2	1.9	
50	13.1	4.83	450.16	7.94	462.93	AT_G42:@.	PMON67211	T2	1.7	
63	12.8	4.78	445.42	7.81	458.00	AT_G56:@.	PMON67211	T2	1.7	
55	12.6	8.32	460.07	7.58	475.98	AT_G47:@.	PMON67211	T2	1.6	
53	13.3	6.43	417.71	6.76	430.91	AT_G45:@.	PMON67211	T2	1.6	
62	12.6	5.36	473.04	6.88	485.28	AT_G55:@.	PMON67211	T2	1.4	
60	12.9	4.87	463.45	5.68	474.00	AT_G53:@.	PMON67211	T2	1.2	

Figure 26 shows T_3 seed δ-tocopherol percentage from two lines expressing MT1 under the control of the napin promoter (pMON67211). Table 9 below shows T_3 seed data from hdt2 mutant lines transformed with pMON67211.

Table 9

Crop	Biotype	Pedigree	mp:aT	mp:gT	mp:dT	total toco.	% delta	Gen
AT	SEED	hdt2:@.0001.0001.	2	280	190	472	40.3	M7
AT	SEED	hdt2:@.0001.0003.	4	263	204	471	43.3	M7
AT	SEED	hdt2:@.0001.0002.	3	262	208	473	44.0	M7
AT	SEED	hdt2:@.0001.0004.	4	271	220	495	44.4	M7
		67211-6					11.0	R2
AT	SEED	67211-HDT2:0006.0005.	4	398	83	485	17.1	R3
AT	SEED	67211-HDT2:0006.0001.	3	438	60	501	12.0	R3
AT	SEED	67211-HDT2:0006.0008.	4	453	59	516	11.4	R3
AT	SEED	67211-HDT2:0006.0002.	3	448	56	507	11.0	R3
AT	SEED	67211-HDT2:0006.0004.	2	417	52	471	11.0	R3

Crop	Biotype	Pedigree	mp:aT	mp:gT	mp:dT	total toco.	% delta	Gen
AT	SEED	67211-HDT2:0006.0007.	3	468	50	521	9.6	R3
AT	SEED	67211-HDT2:0006.0006.	4	464	45	513	8.8	R3
AT	SEED	67211-HDT2:0006.0009.	5	456	42	503	8.3	R3
AT	SEED	67211-HDT2:0006.0003.	4	456	30	490	6.1	R3
		67211-12					12.4	R2
AT	SEED	67211-HDT2:0012.0002.	4	373	102	479	21.3	R3
AT	SEED	67211-HDT2:0012.0009.	3	399	98	500	19.6	R3
AT	SEED	67211-HDT2:0012.0003.	3	397	92	492	18.7	R3
AT	SEED	67211-HDT2:0012.0001.	4	440	66	510	12.9	R3
AT	SEED	67211-HDT2:0012.0008.	2	469	65	536	12.1	R3
AT	SEED	67211-HDT2:0012.0006.	4	438	53	495	10.7	R3
AT	SEED	67211-HDT2:0012.0004.	5	465	54	524	10.3	R3
AT	SEED	67211-HDT2:0012.0005.	5	460	52	517	10.1	R3
AT	SEED	67211-HDT2:0012.0007.	3	458	47	508	9.3	R3

EXAMPLE 9

The CTP-MT1 gene described in example 8 is cloned behind the napin promoter into a binary vector with the ATPT2 gene from *Arabidopsis* and in another double construct with the prenyltransferase (PT) gene (SLR1736 ORF) from *Synechocystis* (described in PCT application WO 0063391).

The MT1 gene is cut out of vector pMON67517 using the restriction enzymes BspHI/PstI and cloned into the PstI/NcoI digested vector backbone of the napin shuttle vector pMON16600, resulting in the formation of pMON67210. The napin cassette from pMON67210, containing the MT1 gene as a translational fusion with the encoded plastid target peptide CTP1 (WO 00/61771) is then cut from this vector with *Not* I and the ends filled in with dNTPs using a Klenow procedure. The resulting fragment is inserted into vectors pMON16602 (digested with *Pme*I) and pCGN10822 (digested with *SnaB*I) to make pMON67213 and pMON67212, respectively (Figures 27 and 28). Vectors pMON16602 and pCGN10822 are described in PCT application WO 0063391.

These double constructs express the MT1 gene and the homogentisate prenyltransferase from either *Arabidopsis* or *Synechocystis* under the control of the napin seed-

specific promoter. The double gene constructs are used to transform *Arabidopsis* and transformed plants are grown to maturity as detailed in Example 2. The resulting T_2 seed is analyzed for total tocopherol content and composition using analytical procedures described in Example 2. Figures 29-32 show total, γ-, δ-, and α- tocopherol levels for various transformed plant lines. Table 10 provides further data from the above-described transformations.

Table 10

ng α toco./mg seed	ng γ toco./mg seed	ng δ toco./mg seed	ng total toco./mg seed	serial number	Pedigree	Construct	
6.28	520.72	13.30	540.30	69000157657	AT00002:@.0321.	Control	For 67212s
5.83	612.04	10.36	628.24	69000157645	AT00002:@.0322.	Control	For 67212s
7.34	621.17	12.62	641.14	69000157633	AT00002:@.0323.	Control	For 67212s
6.48	609.23	13.41	629.12	69000157621	AT00002:@.0324.	Control	For 67212s
6.28	421.10	9.19	436.56	69000157710	AT_G73:@.	PMON67212	
4.72	433.54	7.99	446.24	69000157746	AT_G76:@.	PMON67212	
7.83	570.77	8.77	587.37	69000157758	AT_G77:@.	PMON67212	
7.38	588.65	8.70	604.74	69000157784	AT_G80:@.	PMON67212	
9.56	580.79	14.93	605.28	69000157722	AT_G74:@.	PMON67212	
5.99	605.44	10.38	621.82	69000157847	AT_G86:@.	PMON67212	
7.66	615.03	12.84	635.53	69000157859	AT_G87:@.	PMON67212	
8.29	634.10	9.58	651.97	69000157734	AT_G75:@.	PMON67212	
8.82	628.29	15.95	653.06	69000157809	AT_G82:@.	PMON67212	
7.41	636.96	10.07	654.45	69000157823	AT_G84:@.	PMON67212	
6.64	648.21	10.25	665.10	69000157861	AT_G88:@.	PMON67212	
7.46	624.59	34.85	666.91	69000157811	AT_G83:@.	PMON67212	
8.07	668.83	11.37	688.27	69000157760	AT_G78:@.	PMON67212	
7.96	691.84	11.38	711.18	69000157835	AT_G85:@.	PMON67212	
7.26	705.18	12.01	724.44	69000157796	AT_G81:@.	PMON67212	
7.95	708.29	12.64	728.88	69000157772	AT_G79:@.	PMON67212	
6.95	508.05	11.25	526.25	69000157582	AT00002:@.0328.	Control	For 67213s
8.16	513.84	14.12	536.11	69000157619	AT00002:@.0325.	Control	For 67213s
8.94	547.41	16.60	572.95	69000157607	AT00002:@.0326.	Control	For 67213s
7.83	483.85	15.95	507.63	69000157974	AT_G99:@.	PMON67213	
8.50	488.67	15.92	513.09	69000157671	AT_G101:@.	PMON67213	
7.18	503.50	13.74	524.42	69000157873	AT_G89:@.	PMON67213	

ng α toco./mg seed	ng γ toco./mg seed	ng δ toco./mg seed	ng total toco./mg seed	serial number	Pedigree	Construct	
6.31	511.87	15.83	534.01	69000157950	AT_G97:@.	PMON67213	
7.30	515.26	11.47	534.02	69000157897	AT_G91:@.	PMON67213	
7.11	512.25	19.56	538.92	69000157962	AT_G98:@.	PMON67213	
6.61	525.17	12.82	544.60	69000157900	AT_G92:@.	PMON67213	
7.50	521.38	16.85	545.73	69000157683	AT_G102:@.	PMON67213	
7.87	529.25	11.29	548.41	69000157948	AT_G96:@.	PMON67213	
6.88	523.01	18.83	548.72	69000157912	AT_G93:@.	PMON67213	
7.56	534.21	13.03	554.80	69000157669	AT_G100:@.	PMON67213	
6.79	536.89	12.17	555.86	69000157885	AT_G90:@.	PMON67213	
7.83	535.00	17.97	560.80	69000157936	AT_G95:@.	PMON67213	
8.57	532.53	21.13	562.23	69000157708	AT_G104:@.	PMON67213	
8.15	550.66	18.42	577.23	69000157695	AT_G103:@.	PMON67213	
9.91	560.45	26.66	597.02	69000157924	AT_G94:@.	PMON67213	

What is claimed is:

1. A substantially purified nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2-17, 50, and 85.

2. A substantially purified nucleic acid molecule comprising a nucleic acid sequence that encodes a polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 19-31 and 33-38.

3. A substantially purified nucleic acid molecule comprising as operably linked components: (A) a promoter region which functions in a plant cell to cause the production of an mRNA molecule; (B) a heterologous nucleic acid molecule encoding a polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 19-31 and 33-38.

4. The nucleic acid molecule of claim 3, further comprising a 3' non-translated sequence that functions in said plant cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule.

5. A substantially purified protein comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 19-31 and 33-38.

6. An antibody capable of specifically binding a substantially purified protein with an amino acid sequence selected from the group consisting of SEQ ID NOs: 19-31 and 33-38.

7. A transformed plant comprising an exogenous nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2-17, 50, and 85 and complements thereof.

8. The transformed plant according to claim 7, wherein said plant is selected from the group consisting of canola, corn, *Brassica campestris, Brassica napus*, oilseed rape, rapeseed, soybean, crambe, mustard, castor bean, peanut, sesame, cottonseed, linseed, safflower, oil palm, flax and sunflower.

9. The transformed plant according to claim 8, wherein said plant is canola or oilseed rape.

10. The transformed plant according to claim 8, wherein said plant is soybean or soybean line A3244.

11. The transformed plant according to claim 7, wherein said transformed plant produces a seed with increased α-tocopherol levels relative to a plant with a similar genetic background but lacking said exogenous nucleic acid molecule.

12. The transformed plant according to claim 11, wherein α-tocopherol is the predominant species of tocopherol in said seed

13. The transformed plant of claim 11, wherein said α-tocopherol species comprises greater than about 90% of the total tocopherol content of said seed.

14. The transformed plant according to claim 7, wherein said nucleic acid molecule is operably linked to a promoter.

15. The transformed plant according to claim 14, wherein said promoter is a seed specific promoter.

16. The transformed plant according to claim 15, wherein said promoter is the p7S promoter.

17. The transformed plant according to claim 15, wherein said promoter is the Arcelin 5 promoter or mutants thereof.

18. The transformed plant according to claim 17, wherein said promoter comprises the nucleic acid molecule of SEQ ID NO: 81.

19. The transformed plant according to claim 17, wherein said promoter comprises the nucleic acid molecule of SEQ ID NO: 82.

20. A transformed soybean line A3244 plant, comprising an exogenous nucleic acid molecule comprising a nucleic acid sequence of SEQ ID NO: 2, operably linked to a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of p7S, SEQ ID NO: 81, and SEQ ID NO: 82.

21. A transformed plant having an exogenous nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2-17, 50, and 85 and complements thereof, wherein said transformed plant produces a seed with increased α-tocotrienol levels relative to a plant with a similar genetic background but lacking said exogenous nucleic acid molecule.

22. A transformed plant having an exogenous nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 19-31 and 33-40.

23. The transformed plant according to claim 22, wherein said plant is selected from the group consisting of canola, rapeseed, corn, *Brassica campestris, Brassica*

napus, oilseed rape, soybean, crambe, mustard, castor bean, peanut, sesame, cottonseed, linseed, safflower, oil palm, flax and sunflower.

24. The transformed plant according to claim 23, wherein said plant is canola or oilseed rape.

25. The transformed plant according to claim 23, wherein said plant is soybean.

26. The transformed plant according to claim 20, wherein said transformed plant produces a seed with increased α-tocopherol levels relative to a plant with a similar genetic background but lacking said exogenous nucleic acid molecule.

27. The transformed plant according to claim 26, wherein α-tocopherol is the predominant species of tocopherol in said seed

28. The transformed plant of claim 26, wherein said α-tocopherol species comprises greater than about 90% of the total tocopherol content of said seed.

29. The transformed plant according to claim 20, wherein said nucleic acid molecule is operably linked to a promoter.

30. The transformed plant according to claim 29, wherein said promoter is a seed specific promoter.

31. A transformed plant having an exogenous nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 19-31 and 33-40, wherein said transformed plant produces a seed with increased α-tocotrienol levels relative to a plant with a similar genetic background but lacking said exogenous nucleic acid molecule.

32. A transformed plant having an exogenous nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 46-49.

33. The transformed plant according to claim 32, wherein said plant is selected from the group consisting of canola, rapeseed, corn, *Brassica campestris, Brassica napus,* oilseed rape, soybean, crambe, mustard, castor bean, peanut, sesame, cottonseed, linseed, safflower, oil palm, flax and sunflower.

34. The transformed plant according to claim 33, wherein said plant is canola or oilseed rape.

35. The transformed plant according to claim 33, wherein said plant is soybean.

36. The transformed plant according to claim 32, wherein said transformed plant produces a seed with decreased δ-tocopherol and β-tocopherol levels relative to a plant with a similar genetic background but lacking said exogenous nucleic acid molecule.

37. The transformed plant according to claim 36, wherein γ-tocopherol is the predominant species of tocopherol in said seed

38. The transformed plant of claim 36, wherein said γ-tocopherol species comprises greater than about 90% of the total tocopherol content of said seed.

39. The transformed plant according to claim 32, wherein said nucleic acid molecule is operably linked to a promoter.

40. The transformed plant according to claim 39, wherein said promoter is a seed specific promoter.

41. The transformed plant of claim 32, wherein said transformed plant produces a seed with decreased δ-tocotrienol and β-tocotrienol levels relative to a plant with a similar genetic background but lacking said exogenous nucleic acid molecule.

42. A method for reducing expression of MT1 or GMT in a plant comprising: (A) transforming a plant with a nucleic acid molecule, said nucleic acid molecule having an exogenous promoter region which functions in plant cells to cause the production of a mRNA molecule, wherein said exogenous promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand, wherein said transcribed strand is complementary to a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of: SEQ ID NOs: 2-17, 42-45, 50, and 85; and wherein said transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the plant cells to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA sequence; and (B) growing said transformed plant.

43. A transformed plant comprising a nucleic acid molecule comprising an exogenous promoter region which functions in plant cells to cause the production of a mRNA molecule, wherein said exogenous promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand, wherein said transcribed strand is complementary to a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2-17, 42-45, 50, and 85, and wherein said transcribed nucleic acid molecule is linked to a 3' non-

translated sequence that functions in the plant cells to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA sequence.

44. The transformed plant of claim 43, wherein the expression of MT1 is reduced relative to a plant with a similar genetic background but lacking said exogenous nucleic acid molecule.

45. The transformed plant of claim 43, wherein the expression of GMT is reduced relative to a plant with a similar genetic background but lacking said exogenous nucleic acid molecule.

46. A method for increasing the γ-tocopherol content in a plant comprising:

(A) transforming a plant with a nucleic acid molecule, said nucleic acid molecule having an exogenous promoter region which functions in plant cells to cause the production of a mRNA molecule, wherein said exogenous promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand, wherein said transcribed strand is complementary to a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2-17, 50, and 85; and wherein said transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the plant cells to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA sequence; and (B) growing said transformed plant.

47. A transformed plant comprising:

(A) a first nucleic acid molecule comprising an exogenous promoter region which functions in plant cells to cause the production of a mRNA molecule, wherein said exogenous promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand, wherein said transcribed strand is complementary to a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2-17, 50, and 85, and wherein said transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the plant cells to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA sequence; and (B) a second nucleic acid molecule comprising an exogenous promoter region which functions in plant cells to cause the production of a mRNA molecule, wherein said exogenous promoter region is linked to a nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 42-45.

48. The transformed plant of claim 47, wherein the γ-tocopherol content of said transformed plant is increased relative to a plant with a similar genetic background but lacking said exogenous nucleic acid molecule.

49. A method of producing a plant having a seed with an increased α-tocopherol level comprising: (A) transforming said plant with a nucleic acid molecule, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2-17, 50, and 85; and (B) growing said transformed plant.

50. The method according to claim 49, wherein said plant is selected from the group consisting of canola, rapeseed, corn, *Brassica campestris, Brassica napus,* oilseed rape, soybean, crambe, mustard, castor bean, peanut, sesame, cottonseed, linseed, safflower, oil palm, flax and sunflower.

51. The method according to claim 50, wherein said plant is canola or oilseed rape.

52. The method according to claim 50, wherein said plant is soybean.

53. The method according to claim 49, wherein α-tocopherol is the predominant species of tocopherol in said seed.

54. The method according to claim 49, wherein α-tocopherol comprises greater than about 90% of the total tocopherol content of said seed.

55. A method of producing a plant having a seed with an increased α-tocotrienol level comprising: (A) transforming said plant with a nucleic acid molecule, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2-17, 50, and 85; and (B) growing said transformed plant.

56. A method of producing a plant having a seed with an increased α-tocopherol level comprising: (A) transforming said plant with a nucleic acid molecule, wherein said nucleic acid molecule comprises a sequence encoding an polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 19-31 and 33-40; and (B) growing said transformed plant.

57. The method according to claim 56, wherein said plant is selected from the group consisting of canola, corn, *Brassica campestris, Brassica napus,* oilseed rape, soybean, crambe, mustard, castor bean, peanut, sesame, cottonseed, linseed, safflower, oil palm, flax and sunflower.

58. The method according to claim 57, wherein said transformed plant is canola or oilseed rape.

59. The method according to claim 57, wherein said transformed plant is soybean.

60. A method of producing a plant having a seed with an increased α-tocotrienol level comprising: (A) transforming said plant with a nucleic acid molecule, wherein said nucleic acid molecule comprises a sequence encoding an polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 19-31 and 33-40; and (B) growing said transformed plant.

61. A method of producing a plant having a seed with an increased γ-tocopherol level comprising: (A) transforming said plant with a nucleic acid molecule, wherein said nucleic acid molecule comprises a nucleic acid sequence that encodes an polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 46-49 and (B) growing said transformed plant.

62. A method of producing a plant having a seed with an increased γ-tocopherol level comprising: (A) transforming said plant with a nucleic acid molecule, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 42-45 and (B) growing said transformed plant.

63. The method according to claim 62, wherein said plant is selected from the group consisting of canola, corn, *Brassica campestris, Brassica napus,* oilseed rape, soybean, crambe, mustard, castor bean, peanut, sesame, cottonseed, linseed, safflower, oil palm, flax and sunflower.

64. The method according to claim 63, wherein said transformed plant is canola or oilseed rape.

65. The method according to claim 63, wherein said transformed plant is soybean.

66. The method according to claim 62, wherein γ-tocopherol is the predominant species of tocopherol in said seed.

67. The method according to claim 62, wherein said γ-tocopherol comprises greater than about 90% of the total tocopherol content of said seed.

68. A method of producing a plant having a seed with an increased γ-tocotrienol level comprising: (A) transforming said plant with a nucleic acid molecule, wherein said nucleic acid molecule comprises a nucleic acid sequence that encodes a polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NO: 47 through SEQ ID NO: 49; and (B) growing said transformed plant.

69. A method of producing a plant having a seed with an increased γ-tocotrienol level comprising: (A) transforming said plant with a nucleic acid molecule, wherein

said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 42-46; and (B) growing said transformed plant.

70. A seed derived from a transformed plant having an exogenous nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2-17, 50, and 85, wherein said seed has an increased α-tocopherol level relative to a seed from a plant having a similar genetic background but lacking said exogenous nucleic acid molecule.

71. The seed of claim 70, wherein α-tocopherol is the predominant species of tocopherol in said seed.

72. The seed of claim 70, wherein α-tocopherol comprises greater than about 90% of the total tocopherol content of said seed.

73. A seed derived from a transformed plant having an exogenous nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2-17, 50, and 85, wherein said seed has an increased α-tocotrienol level relative to a seed from a plant having a similar genetic background but lacking said exogenous nucleic acid molecule.

74. Oil derived from a seed of a transformed plant, wherein said transformed plant comprises an exogenous nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 2-17, 42-45, 50, and 85.

75. Feedstock comprising a transformed plant or part thereof, wherein said transformed plant has an exogenous nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2-17, 42-45, 50, and 85.

76. The feedstock of claim 75, wherein the nucleic acid comprises a sequence selected from the group consisting of SEQ ID NOs: 2-17, 50 and 85 wherein said plant produces seeds with increased α-tocopherol levels relative to a plant with a similar genetic background but lacking said exogenous nucleic acid molecule.

77. A meal comprising plant material manufactured from a transformed plant, wherein said transformed plant has an exogenous nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2-17, 42-45, 50, and 85.

78. A seed derived from a transformed plant comprising an exogenous nucleic acid molecule encoding a polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 19-31 and 33-40 wherein said seed has an increased

α-tocopherol level relative to seeds from a plant having a similar genetic background but lacking said exogenous nucleic acid molecule.

79. A seed derived from a transformed plant comprising an exogenous nucleic acid molecule encoding a polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 19-31 and 33-40 wherein said seed has an increased α-tocotrienol level relative to seeds from a plant having a similar genetic background but lacking said exogenous nucleic acid molecule.

80. The seed of claim 79, wherein α-tocopherol is the predominant species of tocopherol in said seed.

81. The seed of claim 79, wherein α-tocopherol comprises greater than about 90% of the total tocopherol content of said seed.

82. Oil derived from a seed of a transformed plant, wherein said transformed plant comprises an exogenous nucleic acid molecule encoding a polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 19-31, 33-40, and 46-49.

83. Feedstock comprising a transformed plant or part thereof, wherein said transformed plant comprises an exogenous nucleic acid molecule encoding a polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 19-31 and 33-40.

84. The feedstock of claim 83, wherein said plant produces seeds with increased α-tocopherol levels relative to a plant with a similar genetic background but lacking said exogenous nucleic acid molecule.

85. A meal comprising plant material manufactured from a transformed plant, wherein said transformed plant comprises an exogenous nucleic acid molecule encoding a polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 19-31, 33-40, and 46-49.

86. A seed derived from a transformed plant comprising an exogenous nucleic acid molecule encoding a polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 46-49, wherein said seed has an increased γ tocopherol level relative to seeds from a plant having a similar genetic background but lacking said exogenous nucleic acid molecule.

87. A seed derived from a transformed plant having an exogenous nucleic acid molecule encoding a polypeptide molecule comprising a sequence selected from the

group consisting of SEQ ID NOs: 46-49, wherein said seed has an increased γ tocotrienol level relative to seeds from a plant having a similar genetic background but lacking said exogenous nucleic acid molecule.

88. Feedstock comprising a transformed plant or part thereof, wherein said transformed plant comprises an exogenous nucleic acid molecule encoding a polypeptide molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 46-49.

89. Meal comprising plant material manufactured from a transformed plant, wherein said transformed plant has an exogenous nucleic acid molecule encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 46-49.

90. A host cell comprising a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2-17, 42-45, 50, and 85 and complements thereof.

91. The host cell according to claim 90, wherein said cell is a bacterial cell.

92. The host cell according to claim 90, wherein said cell is an *Agrobacterium tumefaciens* or *E. coli* cell.

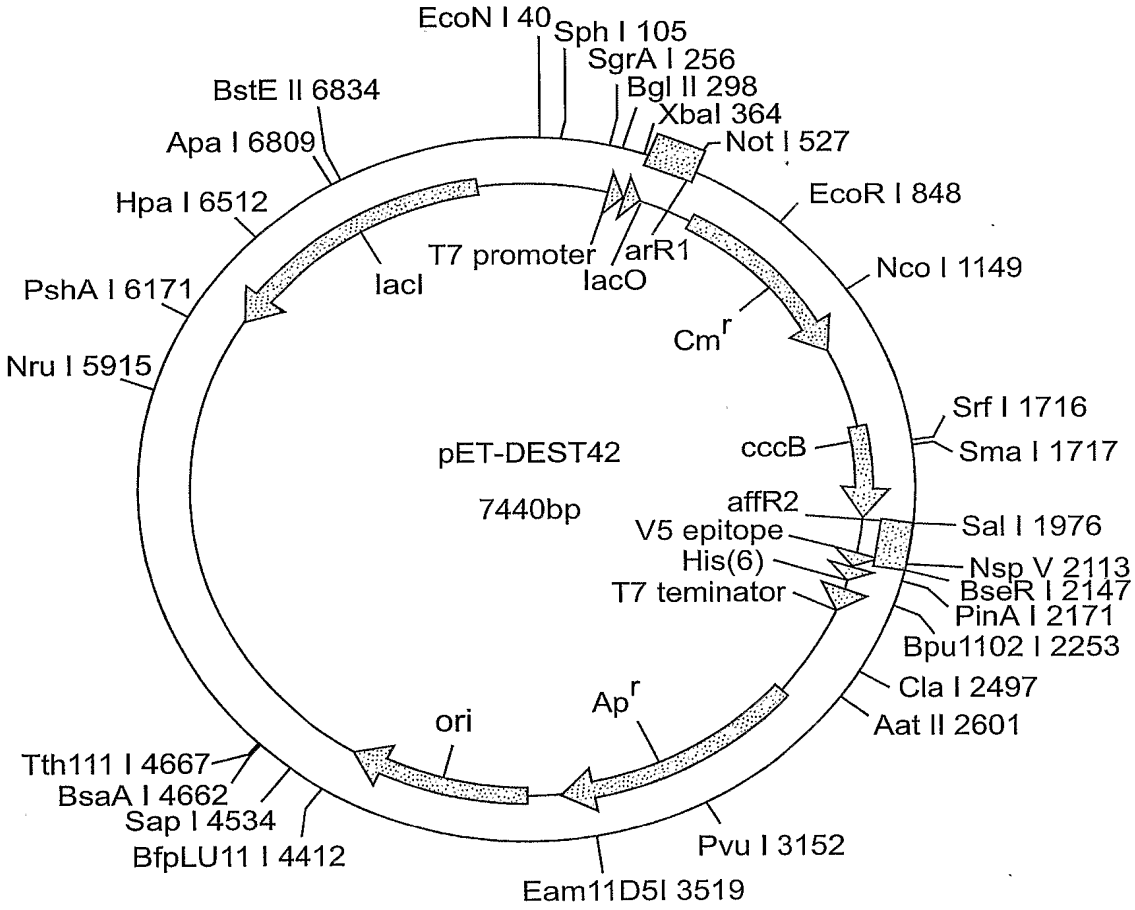
93. A transformed plant comprising an introduced first nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 19-31, 33-38, and 39-41, and an introduced second nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA, slr1736, ATPT2, dxs, dxr, GGPPS, HPPD, GMT, MT1, tMT2, AANT1, slr 1737*, and an antisense construct for homogentisic acid dioxygenase.

94. A transformed plant comprising an introduced first nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 46-49, and an introduced second nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA, slr1736, ATPT2, dxs, dxr, GGPPS, HPPD, GMT, MT1, tMT2, AANT1, slr 1737*, and an antisense construct for homogentisic acid dioxygenase.

95. A plant comprising an introduced nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of 42-45, wherein said transformed plant produces a seed having increased total tocopherol relative to seed of a plant with a similar genetic background but lacking said introduced nucleic acid molecule.

96. A plant comprising an introduced nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of 2-17, 50, 85, wherein said transformed plant produces a seed having increased total tocopherol relative to seed of a plant with a similar genetic background but lacking said introduced nucleic acid molecule.

97. A plant comprising a first introduced nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of 2-17, 50, and 85 and a second introduced nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of 42-45, wherein said transformed plant produces a seed having increased total tocopherol relative to seed of a plant with a similar genetic background but lacking both said introduced first nucleic acid molecule and said introduced second nucleic acid molecule.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Sal I 1976
Nsp V 2113
BseR I 2147
PinA I 2171
Bpu1102 I 2253
Cla I 2497
Aat II 2601
Pvu I 3152
Eam11D5I 3519
Tth111 I 4667
BsaA I 4662
Sap I 4534
BfpLU11 I 4412
Nru I 5915
PshA I 6171
Hpa I 6512
Apa I 6809
BstE II 6834
T7 promoter
lacO
arR1
lacI
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affR2
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T7 teminator
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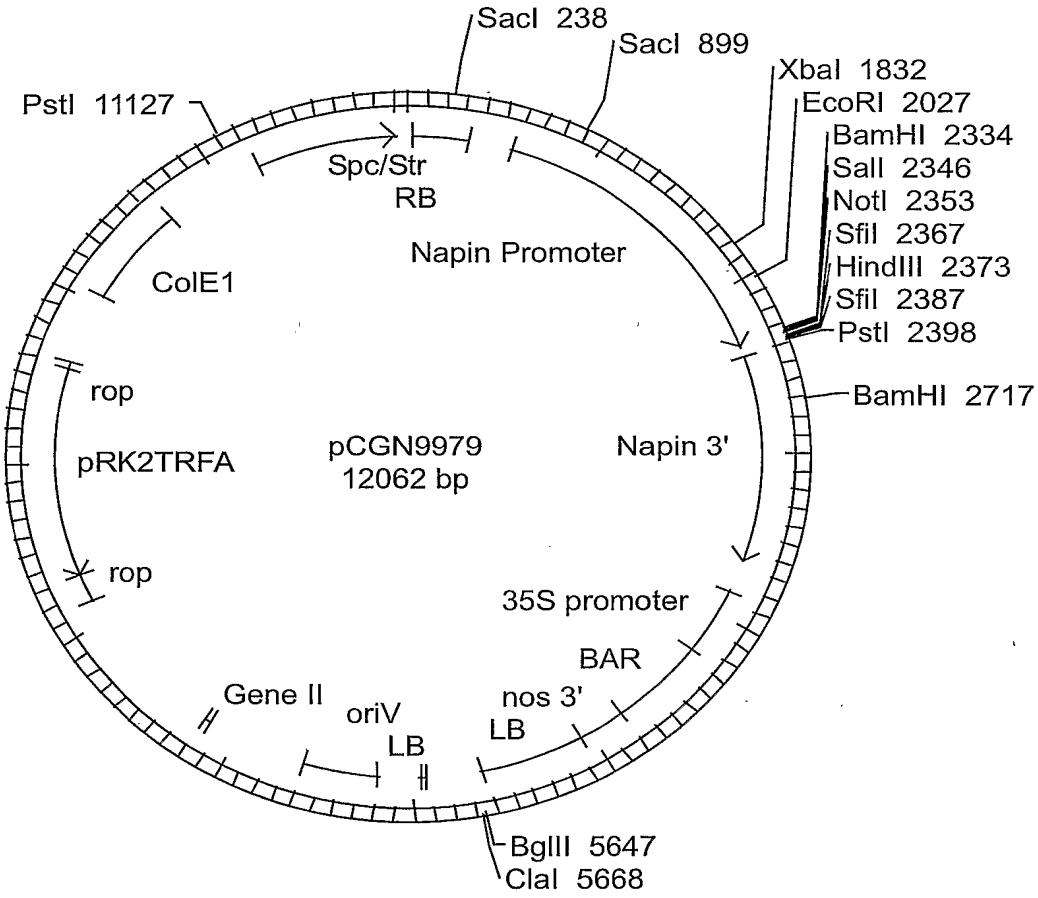
FIG. 1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PstI 11127
BamHI 2717
Spc/Str
RB
Napin Promoter
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rop
pRK2TRFA
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nos 3'
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LB
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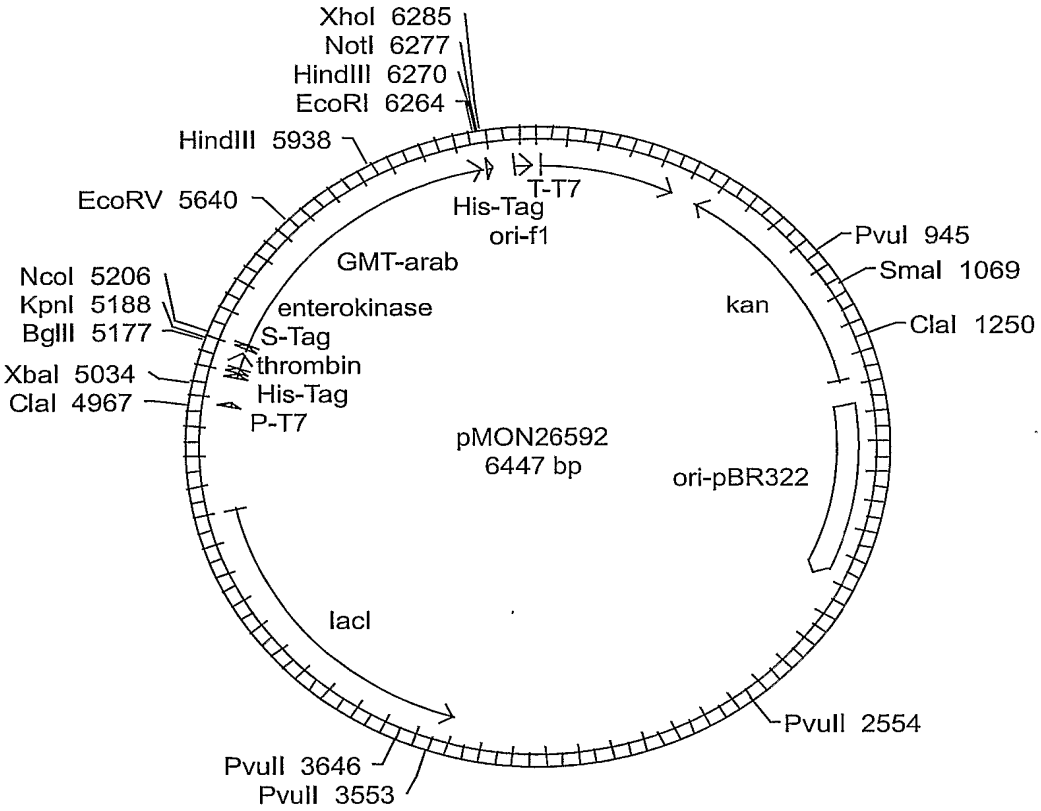
ClaI 5668

FIG. 2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ClaI 4967
T-T7
His-Tag
ori-f1
GMT-arab
enterokinase
S-Tag
thrombin
His-Tag
P-T7
PvuI 945
SmaI 1069
ClaI 1250
kan
pMON26592
6447 bp
ori-pBR322
lacI
PvuII 2554
PvuII 3646
PvuII 3553

FIG. 3

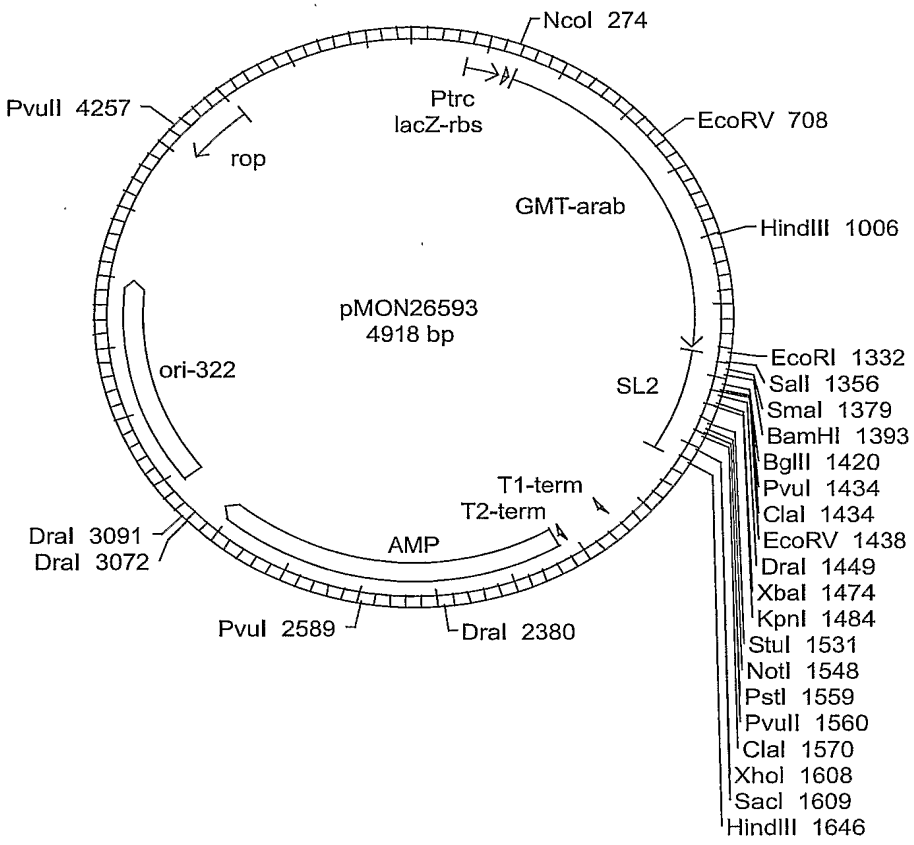
NcoI 274
Ptrc
lacZ-rbs
EcoRV 708
PvuII 4257
rop
GMT-arab
HindIII 1006
pMON26593
4918 bp
ori-322
SL2
EcoRI 1332
SalI 1356
SmaI 1379
BamHI 1393
BglII 1420
PvuI 1434
ClaI 1434
EcoRV 1438
DraI 1449
XbaI 1474
KpnI 1484
StuI 1531
NotI 1548
PstI 1559
PvuII 1560
ClaI 1570
XhoI 1608
SacI 1609
HindIII 1646
T1-term
T2-term
AMP
DraI 3091
DraI 3072
PvuI 2589
DraI 2380

FIG. 4

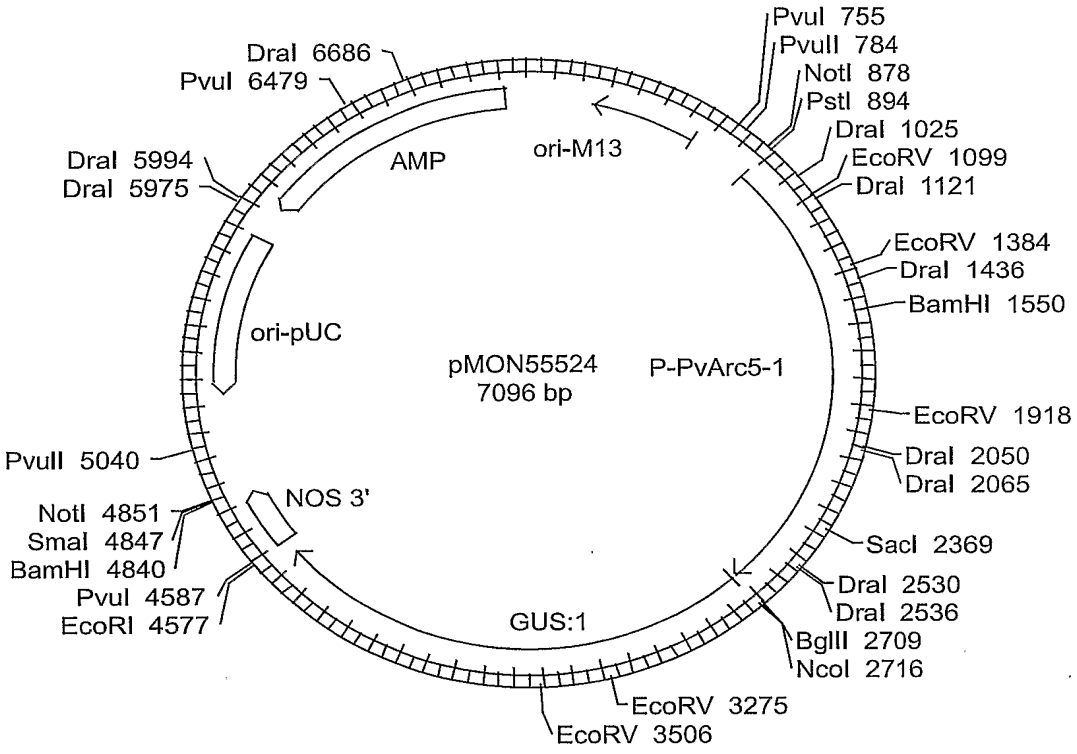
PvuI 755
PvuII 784
NotI 878
PstI 894
DraI 1025
EcoRV 1099
DraI 1121
EcoRV 1384
DraI 1436
BamHI 1550
EcoRV 1918
DraI 2050
DraI 2065
SacI 2369
DraI 2530
DraI 2536
BglII 2709
NcoI 2716
EcoRV 3275
EcoRV 3506
PvuII 5040
NotI 4851
SmaI 4847
BamHI 4840
PvuI 4587
EcoRI 4577
DraI 6686
PvuI 6479
DraI 5994
DraI 5975
AMP
ori-M13
ori-pUC
NOS 3'
GUS:1
pMON55524
7096 bp
P-PvArc5-1

FIG. 5

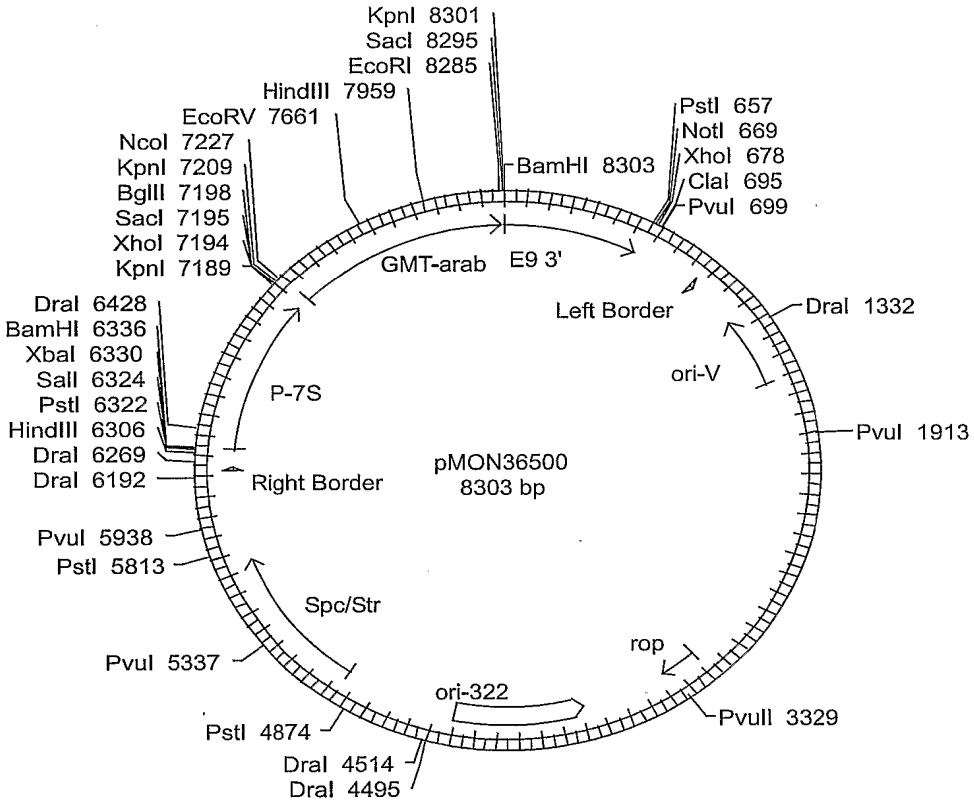
KpnI 8301
SacI 8295
EcoRI 8285
HindIII 7959
EcoRV 7661
NcoI 7227
KpnI 7209
BglII 7198
SacI 7195
XhoI 7194
KpnI 7189
BamHI 8303
PstI 657
NotI 669
XhoI 678
ClaI 695
PvuI 699
GMT-arab
E9 3'
Left Border
DraI 1332
ori-V
PvuI 1913
DraI 6428
BamHI 6336
XbaI 6330
SalI 6324
PstI 6322
HindIII 6306
DraI 6269
DraI 6192
P-7S
Right Border
pMON36500
8303 bp
PvuI 5938
PstI 5813
Spc/Str
PvuI 5337
rop
ori-322
PvuII 3329
PstI 4874
DraI 4514
DraI 4495

FIG. 6

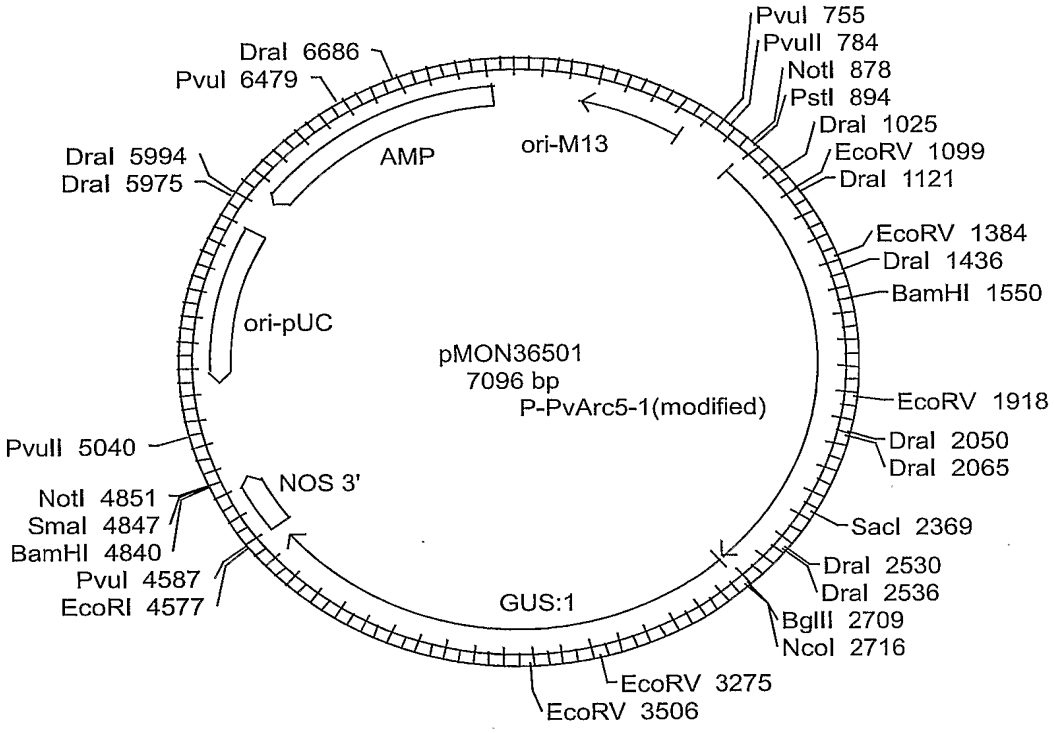
DraI 6686
PvuI 6479
DraI 5994
DraI 5975
AMP
ori-M13
PvuI 755
PvuII 784
NotI 878
PstI 894
DraI 1025
EcoRV 1099
DraI 1121
EcoRV 1384
DraI 1436
BamHI 1550
ori-pUC
pMON36501
7096 bp
P-PvArc5-1(modified)
EcoRV 1918
DraI 2050
DraI 2065
PvuII 5040
NOS 3'
NotI 4851
SmaI 4847
BamHI 4840
PvuI 4587
EcoRI 4577
SacI 2369
DraI 2530
DraI 2536
BglII 2709
NcoI 2716
GUS:1
EcoRV 3275
EcoRV 3506

FIG. 7

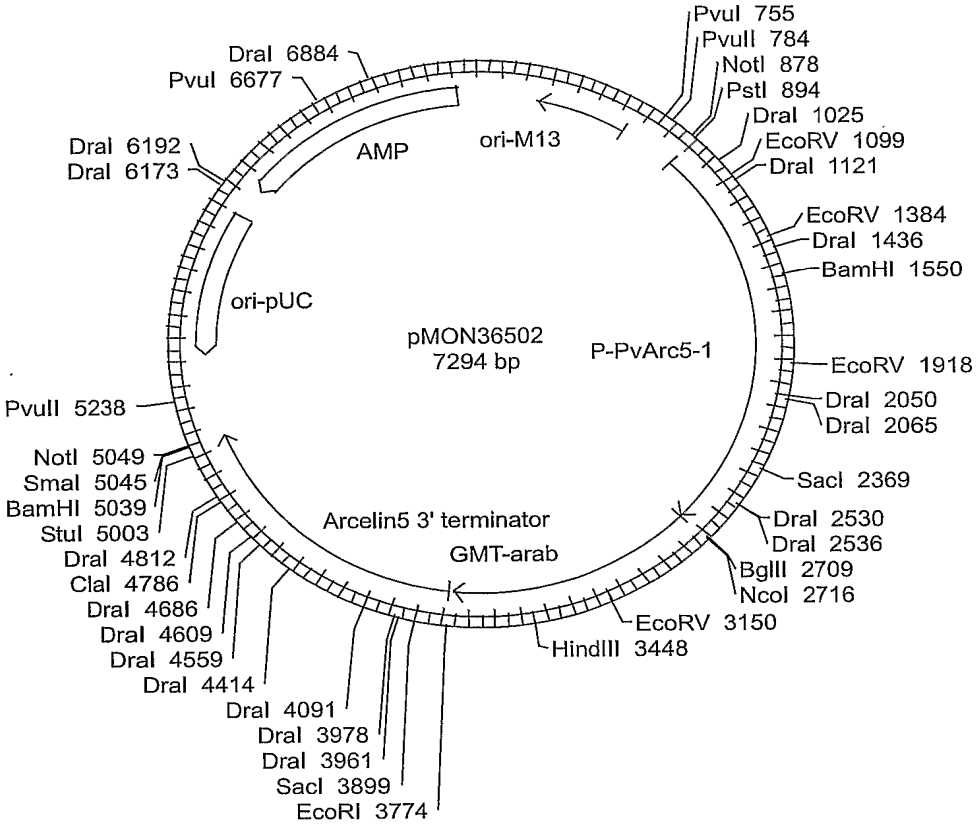
PvuI 755
PvuII 784
NotI 878
PstI 894
DraI 1025
EcoRV 1099
DraI 1121
EcoRV 1384
DraI 1436
BamHI 1550
EcoRV 1918
DraI 2050
DraI 2065
SacI 2369
DraI 2530
DraI 2536
BglII 2709
NcoI 2716
EcoRV 3150
HindIII 3448
DraI 6884
PvuI 6677
DraI 6192
DraI 6173
AMP
ori-M13
ori-pUC
pMON36502
7294 bp
P-PvArc5-1
PvuII 5238
NotI 5049
SmaI 5045
BamHI 5039
StuI 5003
DraI 4812
ClaI 4786
DraI 4686
DraI 4609
DraI 4559
DraI 4414
DraI 4091
DraI 3978
DraI 3961
SacI 3899
EcoRI 3774
Arcelin5 3' terminator
GMT-arab

FIG. 8

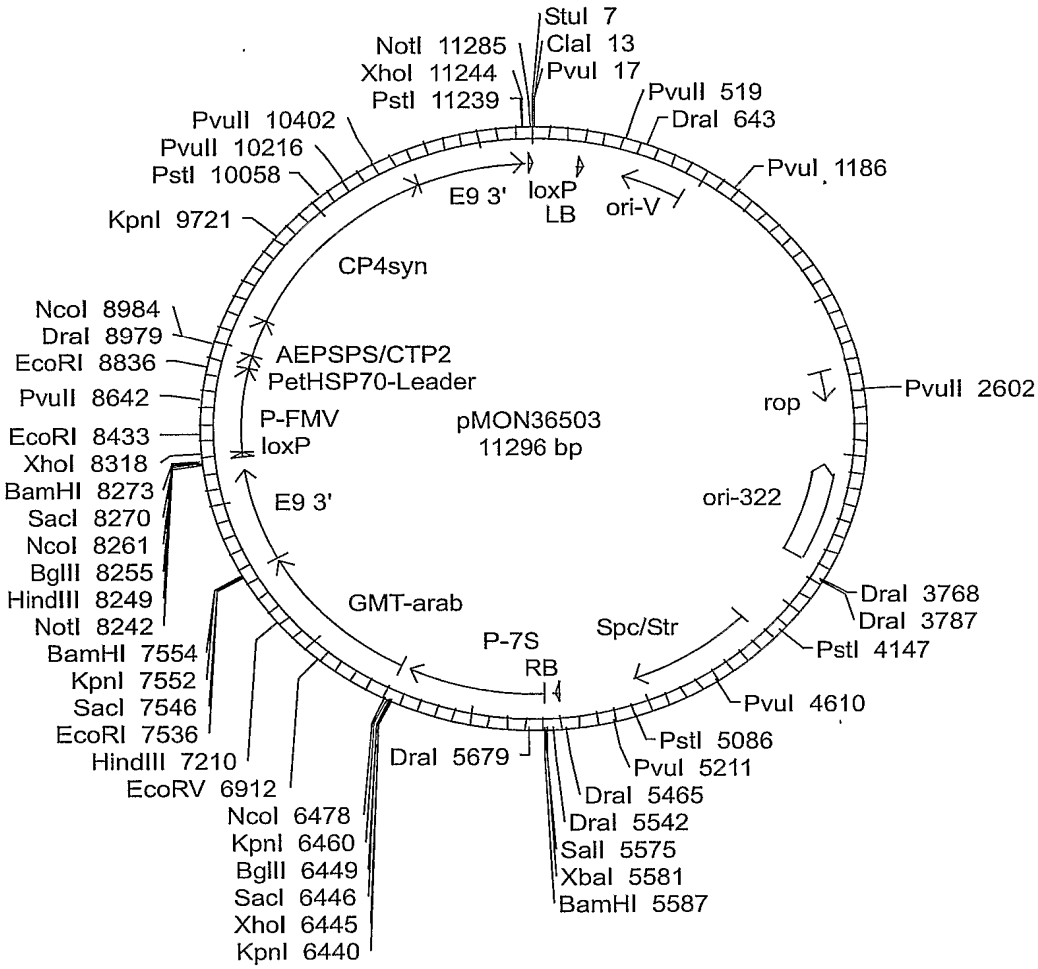
StuI 7
ClaI 13
PvuI 17
NotI 11285
XhoI 11244
PstI 11239
PvuII 519
DraI 643
PvuII 10402
PvuII 10216
PstI 10058
PvuI 1186
E9 3'
loxP
LB
ori-V
KpnI 9721
CP4syn
NcoI 8984
DraI 8979
EcoRI 8836
AEPSPS/CTP2
PetHSP70-Leader
PvuII 8642
P-FMV
loxP
pMON36503
11296 bp
PvuII 2602
rop
EcoRI 8433
XhoI 8318
BamHI 8273
SacI 8270
NcoI 8261
BglII 8255
HindIII 8249
NotI 8242
E9 3'
ori-322
GMT-arab
DraI 3768
DraI 3787
PstI 4147
Spc/Str
P-7S
RB
PvuI 4610
BamHI 7554
KpnI 7552
SacI 7546
EcoRI 7536
HindIII 7210
EcoRV 6912
NcoI 6478
KpnI 6460
BglII 6449
SacI 6446
XhoI 6445
KpnI 6440
DraI 5679
PstI 5086
PvuI 5211
DraI 5465
DraI 5542
SalI 5575
XbaI 5581
BamHI 5587

FIG. 9

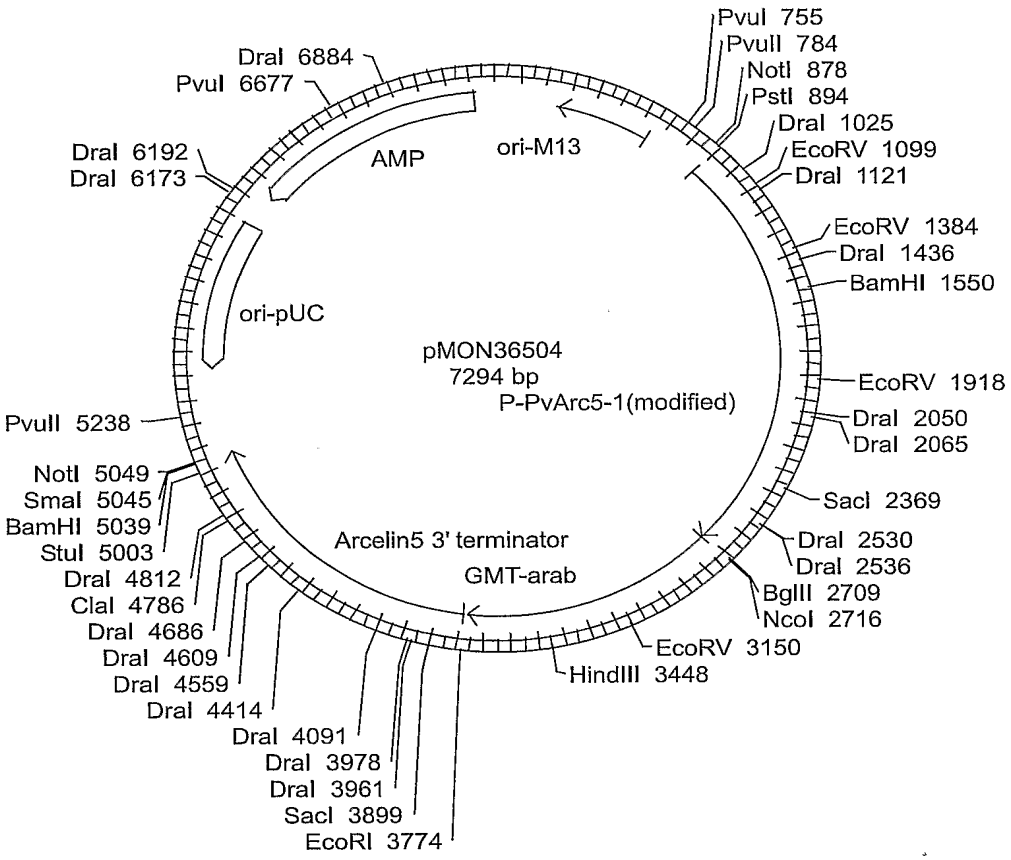
PvuI 755
PvuII 784
NotI 878
PstI 894
DraI 1025
EcoRV 1099
DraI 1121
EcoRV 1384
DraI 1436
BamHI 1550
EcoRV 1918
DraI 2050
DraI 2065
SacI 2369
DraI 2530
DraI 2536
BglII 2709
NcoI 2716
EcoRV 3150
HindIII 3448
DraI 4091
DraI 3978
DraI 3961
SacI 3899
EcoRI 3774
DraI 4414
DraI 4559
DraI 4609
DraI 4686
ClaI 4786
DraI 4812
StuI 5003
BamHI 5039
SmaI 5045
NotI 5049
PvuII 5238
DraI 6173
DraI 6192
PvuI 6677
DraI 6884
AMP
ori-M13
ori-pUC
pMON36504
7294 bp
P-PvArc5-1(modified)
Arcelin5 3' terminator
GMT-arab

FIG. 10

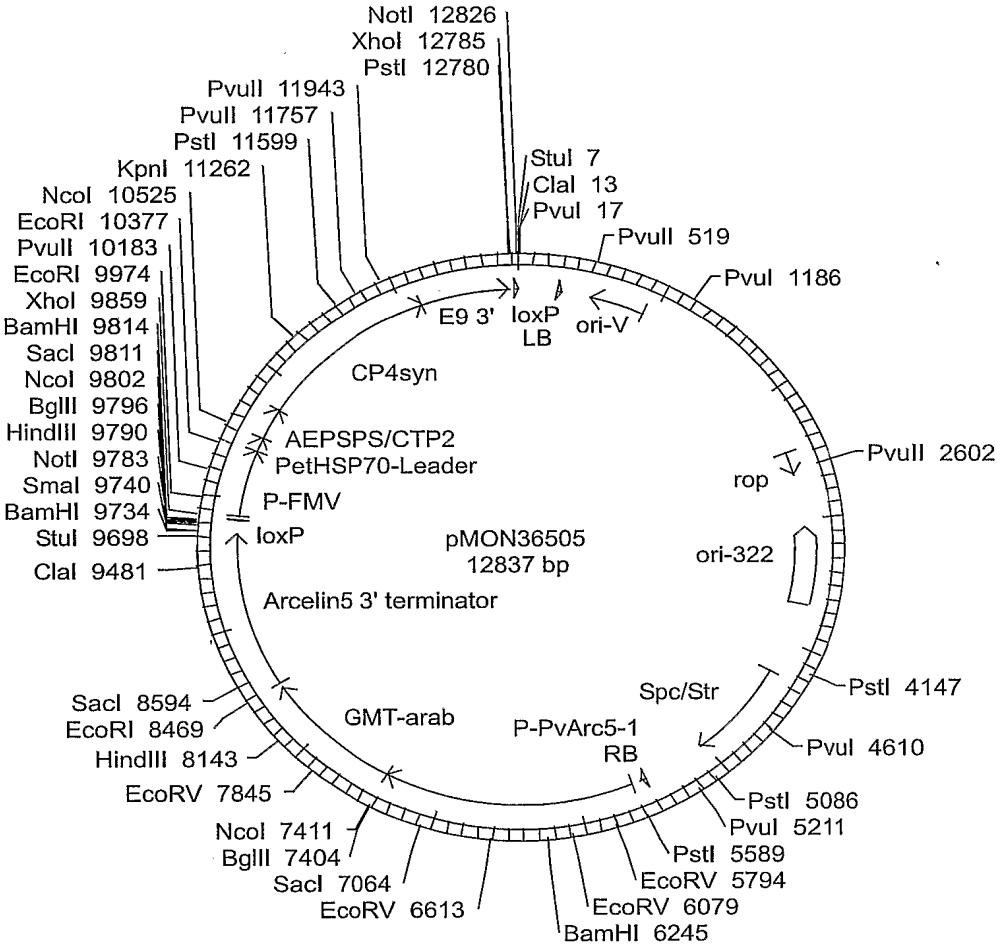
NotI 12826
XhoI 12785
PstI 12780
PvuII 11943
PvuII 11757
PstI 11599
KpnI 11262
NcoI 10525
EcoRI 10377
PvuII 10183
EcoRI 9974
XhoI 9859
BamHI 9814
SacI 9811
NcoI 9802
BglII 9796
HindIII 9790
NotI 9783
SmaI 9740
BamHI 9734
StuI 9698
ClaI 9481
StuI 7
ClaI 13
PvuI 17
PvuII 519
PvuI 1186
E9 3'
loxP
LB
ori-V
CP4syn
AEPSPS/CTP2
PetHSP70-Leader
P-FMV
loxP
pMON36505
12837 bp
Arcelin5 3' terminator
PvuII 2602
rop
ori-322
PstI 4147
Spc/Str
PvuI 4610
P-PvArc5-1
RB
PstI 5086
PvuI 5211
PstI 5589
EcoRV 5794
EcoRV 6079
BamHI 6245
EcoRV 6613
SacI 7064
BglII 7404
NcoI 7411
EcoRV 7845
HindIII 8143
EcoRI 8469
SacI 8594
GMT-arab

FIG. 11

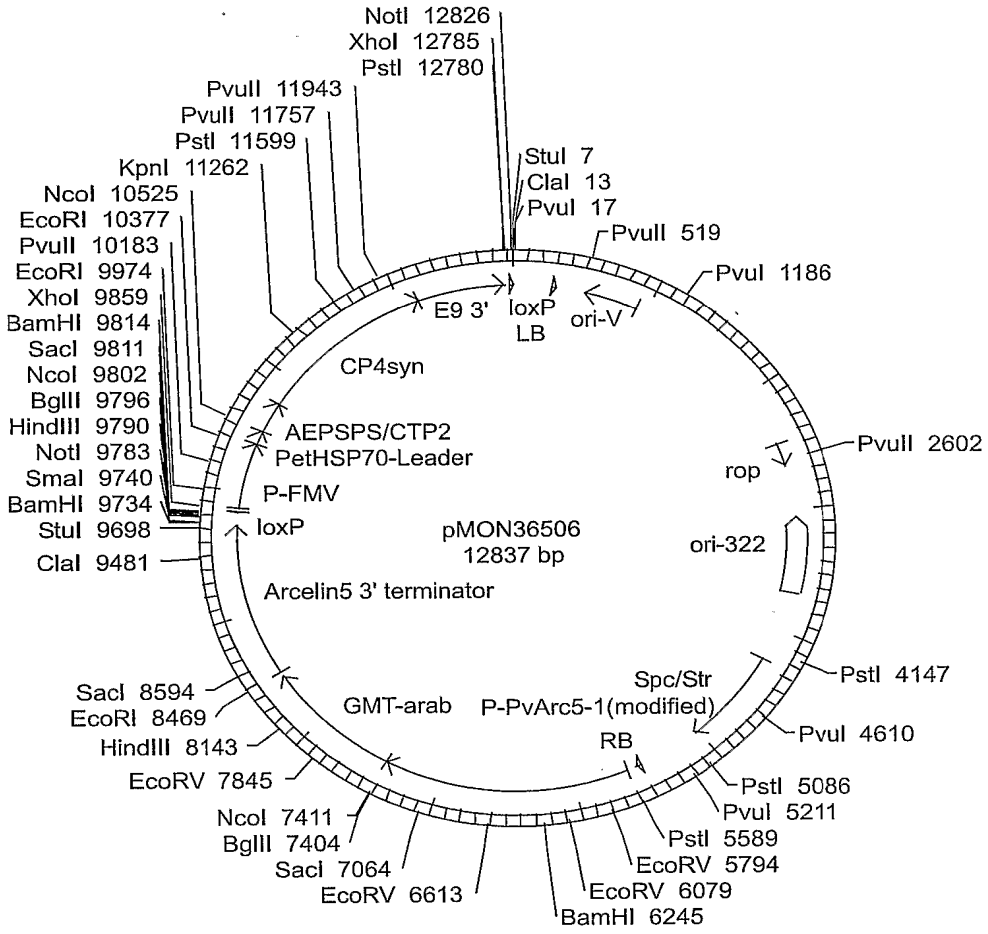
NotI 12826
XhoI 12785
PstI 12780
PvuII 11943
PvuII 11757
PstI 11599
KpnI 11262
NcoI 10525
EcoRI 10377
PvuII 10183
EcoRI 9974
XhoI 9859
BamHI 9814
SacI 9811
NcoI 9802
BglII 9796
HindIII 9790
NotI 9783
SmaI 9740
BamHI 9734
StuI 9698
ClaI 9481
StuI 7
ClaI 13
PvuI 17
PvuII 519
PvuI 1186
PvuII 2602
PstI 4147
PvuI 4610
PstI 5086
PvuI 5211
PstI 5589
EcoRV 5794
EcoRV 6079
BamHI 6245
EcoRV 6613
SacI 7064
NcoI 7411
BglII 7404
EcoRV 7845
HindIII 8143
EcoRI 8469
SacI 8594
E9 3'
loxP
LB
ori-V
CP4syn
AEPSPS/CTP2
PetHSP70-Leader
P-FMV
loxP
Arcelin5 3' terminator
pMON36506
12837 bp
rop
ori-322
Spc/Str
P-PvArc5-1(modified)
RB
GMT-arab

FIG. 12

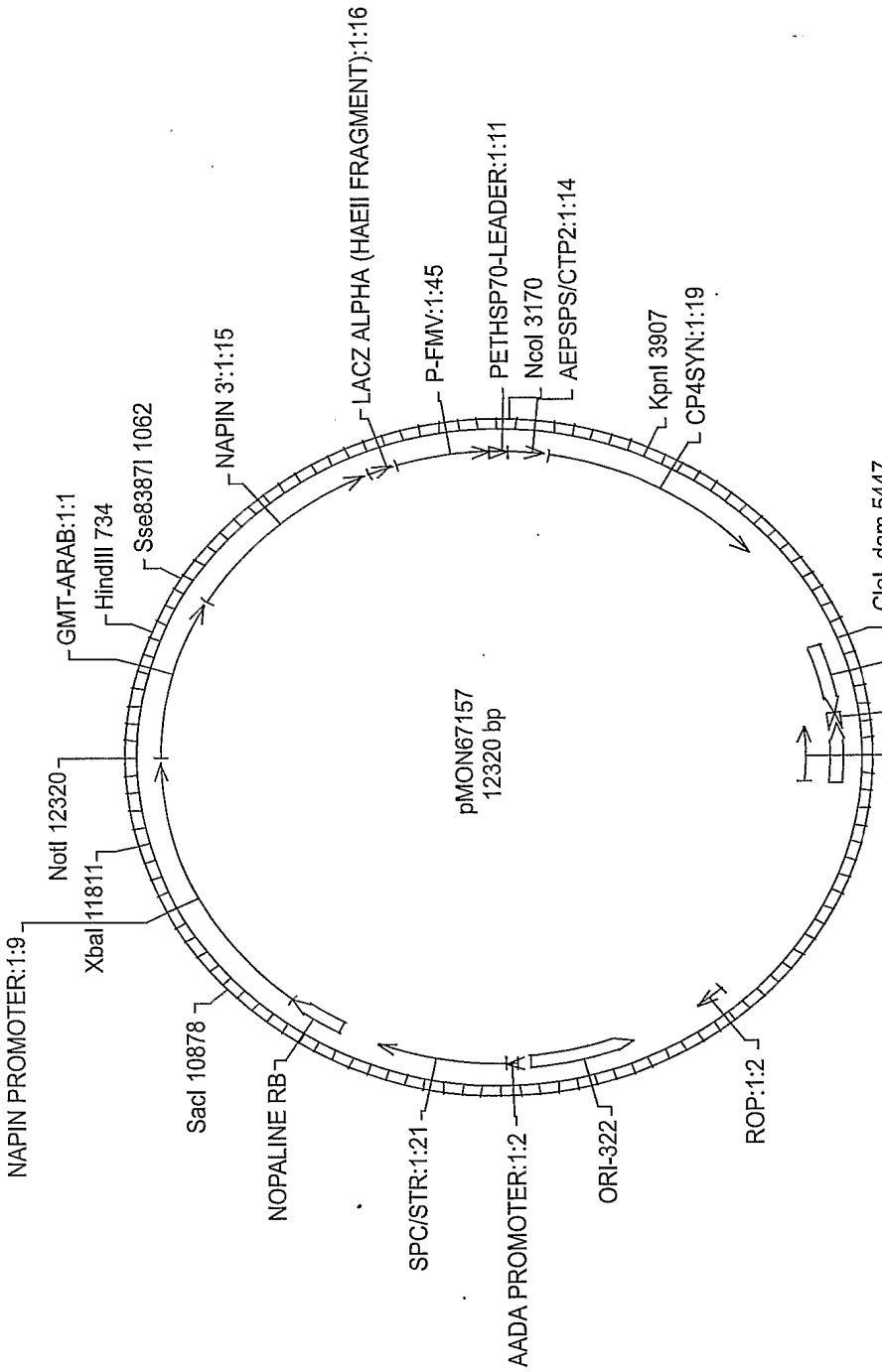
pMON67157
12320 bp
NAPIN PROMOTER:1:9
NotI 12320
XbaI 11811
SacI 10878
NOPALINE RB
SPC/STR:1:21
AADA PROMOTER:1:2
ORI-322
ROP:1:2
ori-V:1:1103
ORI-V
T-R12.1
OCTOPINE LB
ClaI_dam 5447
KpnI 3907
CP4SYN:1:19
AEPSPS/CTP2:1:14
NcoI 3170
PETHSP70-LEADER:1:11
P-FMV:1:45
LACZ ALPHA (HAEII FRAGMENT):1:16
NAPIN 3:1:15
Sse8387I 1062
HindIII 734
GMT-ARAB:1:1

FIG. 13

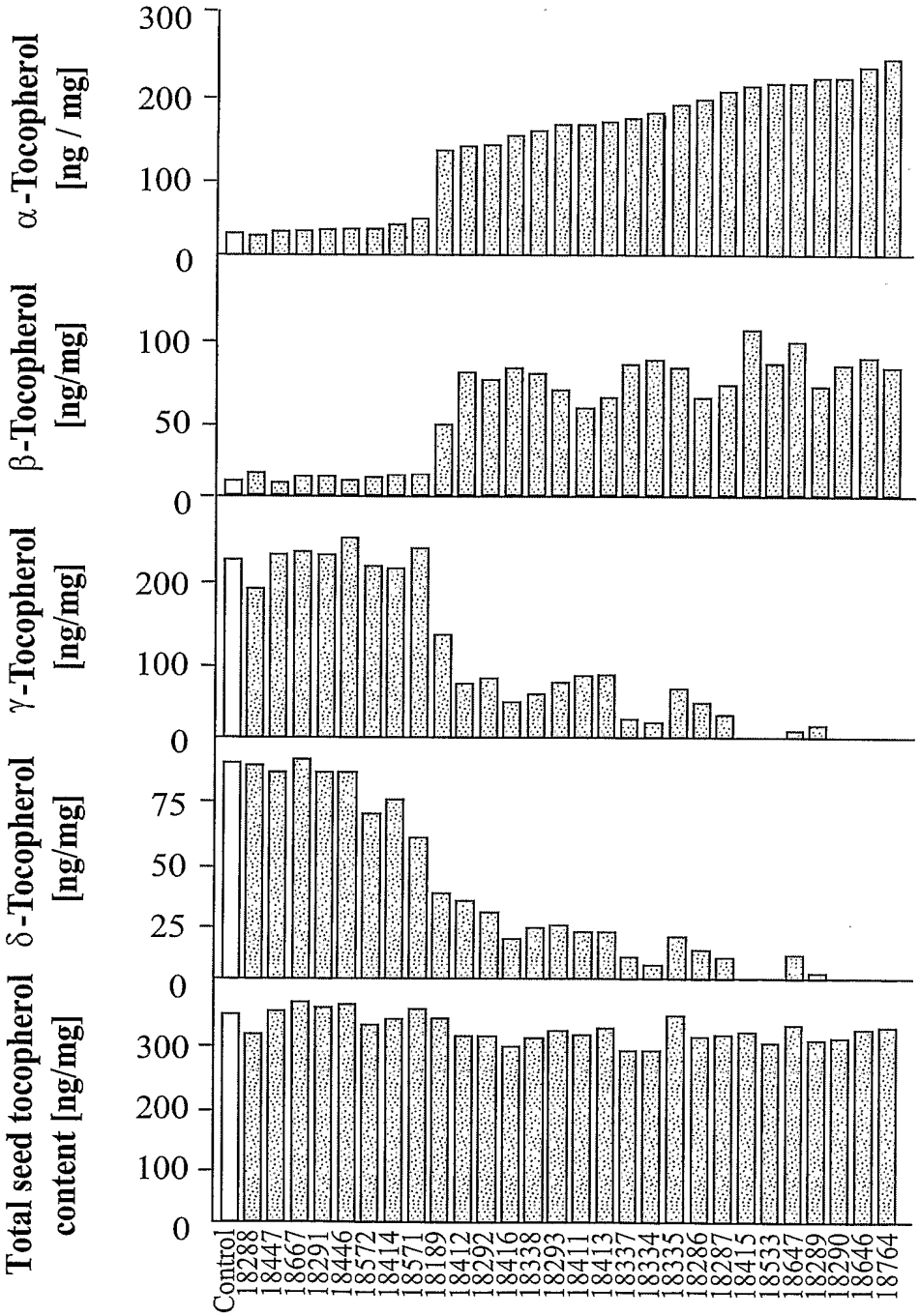
α-Tocopherol [ng / mg]
β-Tocopherol [ng/mg]
γ-Tocopherol [ng/mg]
δ-Tocopherol [ng/mg]
Total seed tocopherol content [ng/mg]
300
200
100
0
100
50
0
200
100
0
75
50
25
0
300
200
100
0
Control
18288
18447
18667
18291
18446
18572
18414
18571
18189
18412
18292
18416
18338
18293
18411
18413
18337
18334
18335
18286
18287
18415
18533
18647
18289
18290
18646
18764

FIG. 14

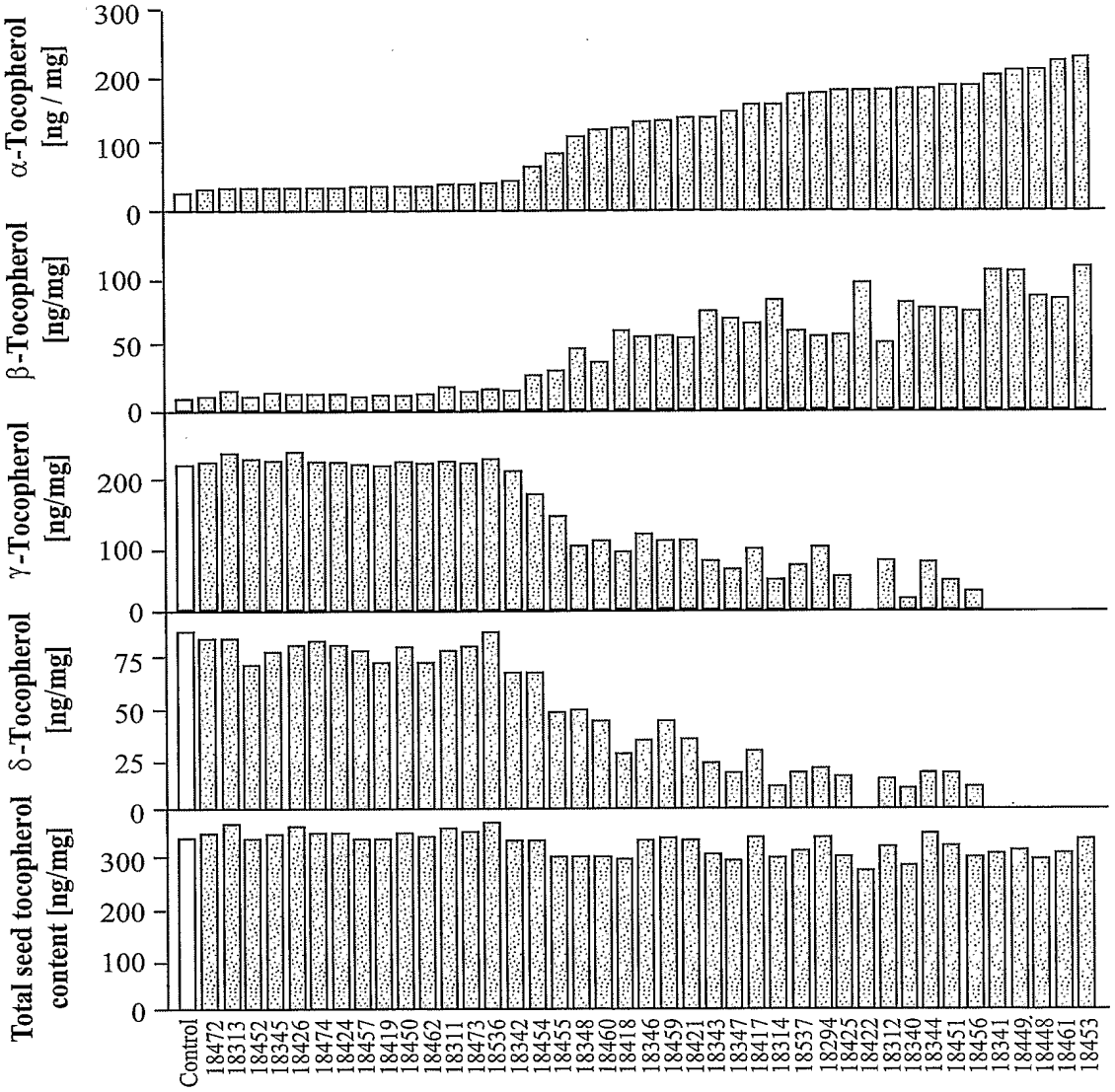
α-Tocopherol [ng / mg]
300
200
100
0
β-Tocopherol [ng/mg]
100
50
0
γ-Tocopherol [ng/mg]
200
100
0
δ-Tocopherol [ng/mg]
75
50
25
0
Total seed tocopherol content [ng/mg]
300
200
100
0
Control
18472
18313
18452
18345
18426
18474
18424
18457
18419
18450
18462
18311
18473
18536
18342
18454
18455
18348
18460
18418
18346
18459
18421
18343
18347
18417
18314
18537
18294
18425
18422
18312
18340
18344
18451
18456
18341
18449
18448
18461
18453

FIG. 15

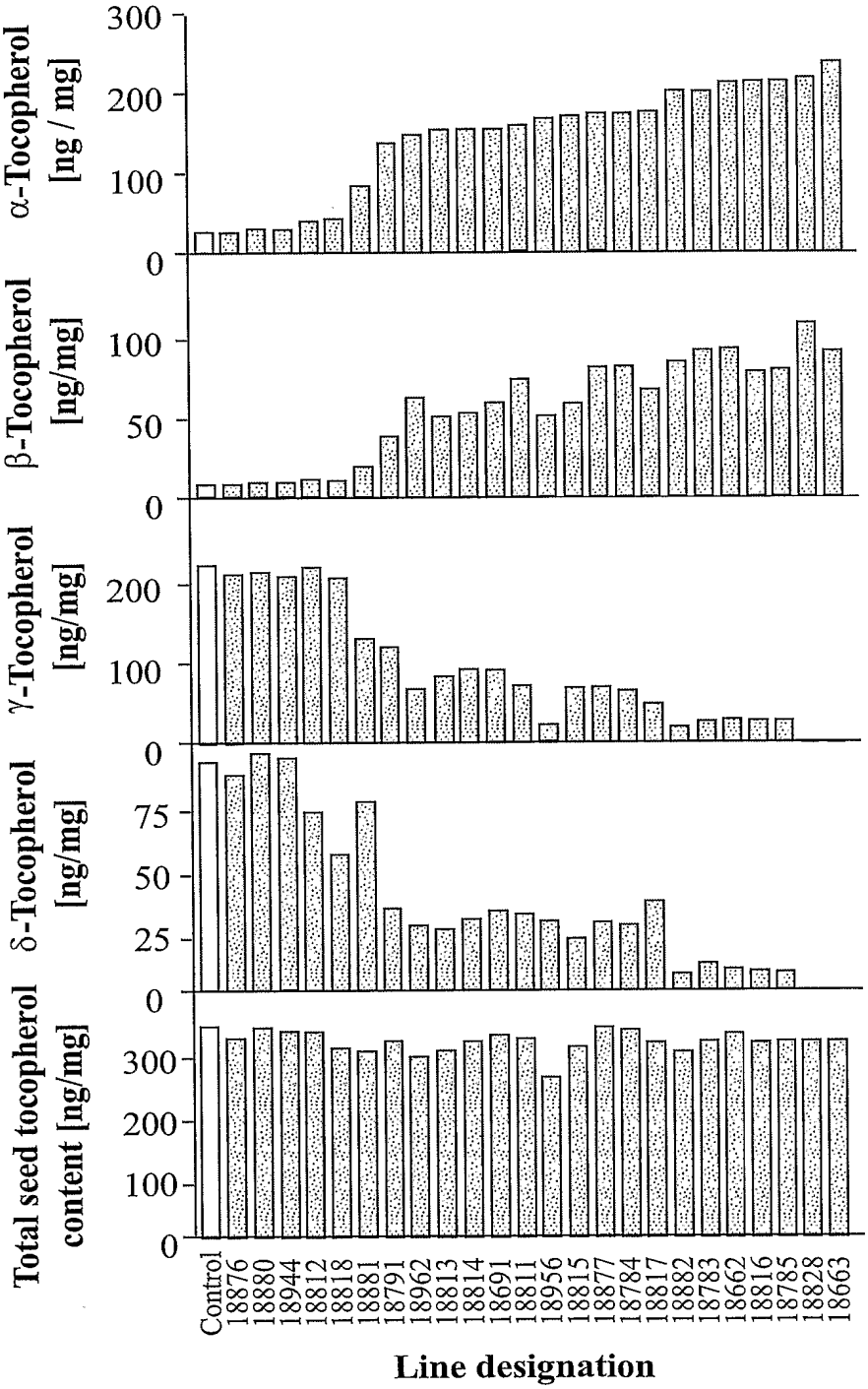
α-Tocopherol [ng / mg]
300
200
100
0
β-Tocopherol [ng/mg]
100
50
0
γ-Tocopherol [ng/mg]
200
100
0
δ-Tocopherol [ng/mg]
75
50
25
0
Total seed tocopherol content [ng/mg]
300
200
100
0
Control
18876
18880
18944
18812
18818
18881
18791
18962
18813
18814
18691
18811
18956
18815
18877
18784
18817
18882
18783
18662
18816
18785
18828
18663
Line designation

FIG. 16

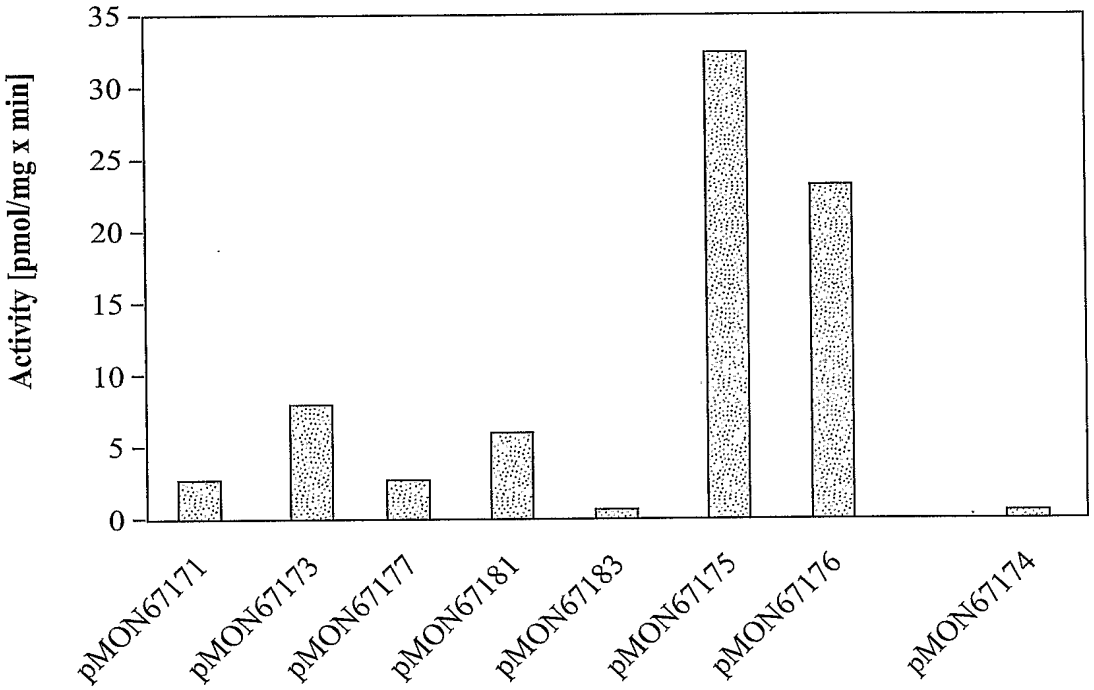
Activity [pmol/mg x min]
35
30
25
20
15
10
5
0
pMON67171
pMON67173
pMON67177
pMON67181
pMON67183
pMON67175
pMON67176
pMON67174

FIG. 17

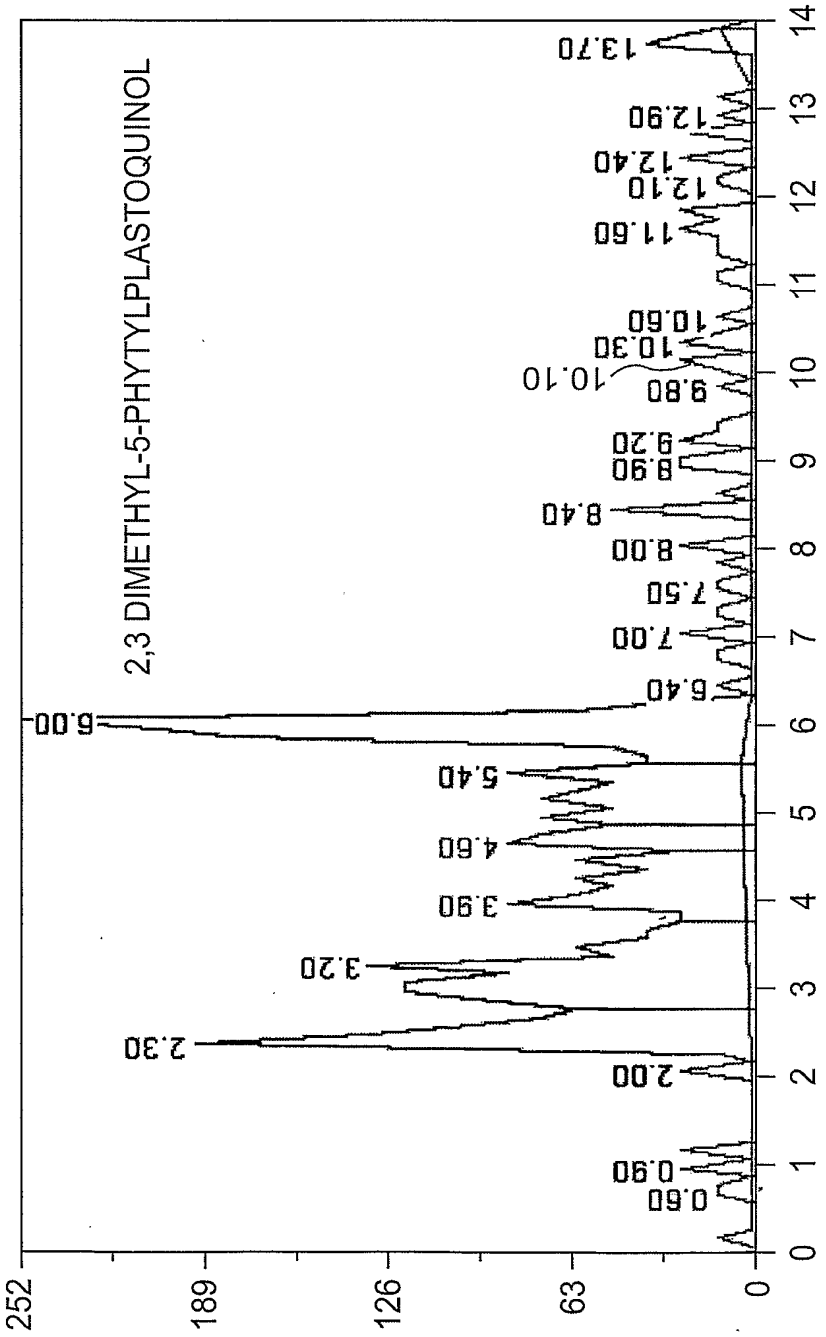
2,3 DIMETHYL-5-PHYTYLPLASTOQUINOL
252
189
126
63
0
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
0.60
0.90
2.00
2.30
3.20
3.90
4.60
5.40
6.00
6.40
7.00
7.50
8.00
8.40
8.90
9.20
9.80
10.10
10.30
10.60
11.60
12.10
12.40
12.90
13.70

FIG. 18

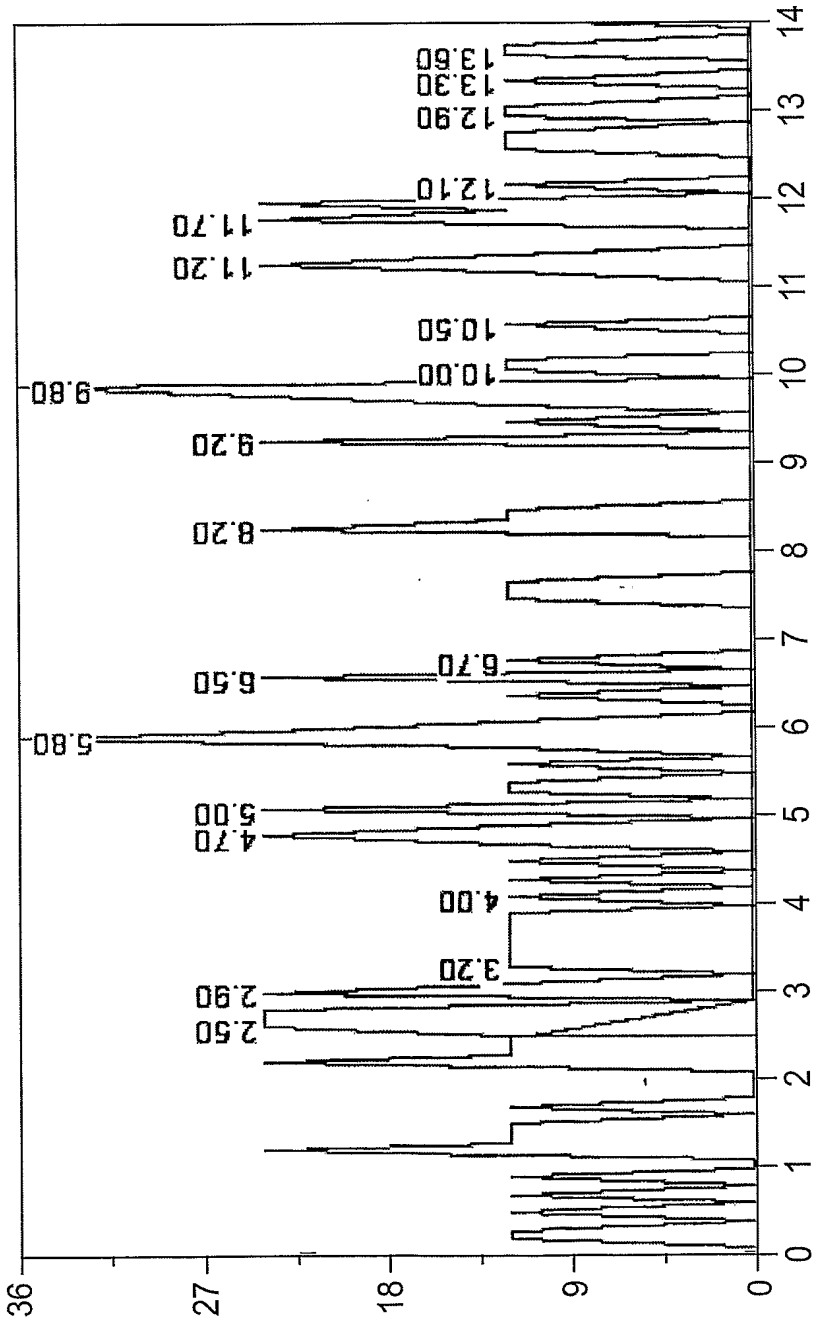
13.60
13.30
12.90
12.10
11.70
11.20
10.50
10.00
9.80
9.20
8.20
6.70
6.50
5.80
5.00
4.70
4.00
3.20
2.90
2.50
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
36
27
18
9
0

FIG. 19

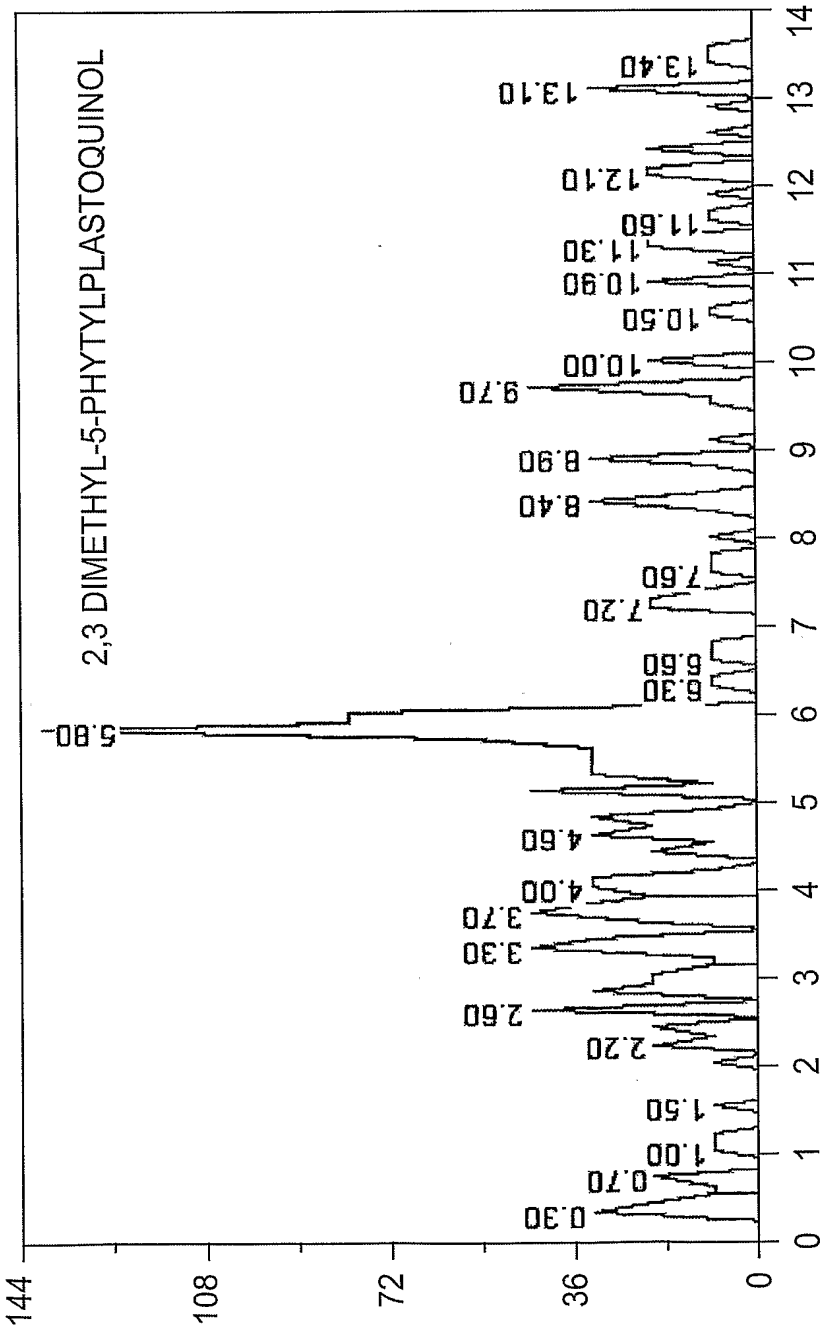
2,3 DIMETHYL-5-PHYTYLPLASTOQUINOL
144
108
72
36
0
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
0.30
0.70
1.00
1.50
2.20
2.60
3.30
3.70
4.00
4.60
5.80
6.30
6.60
7.20
7.60
8.40
8.90
9.70
10.00
10.50
10.90
11.30
11.60
12.10
13.10
13.40

FIG. 20

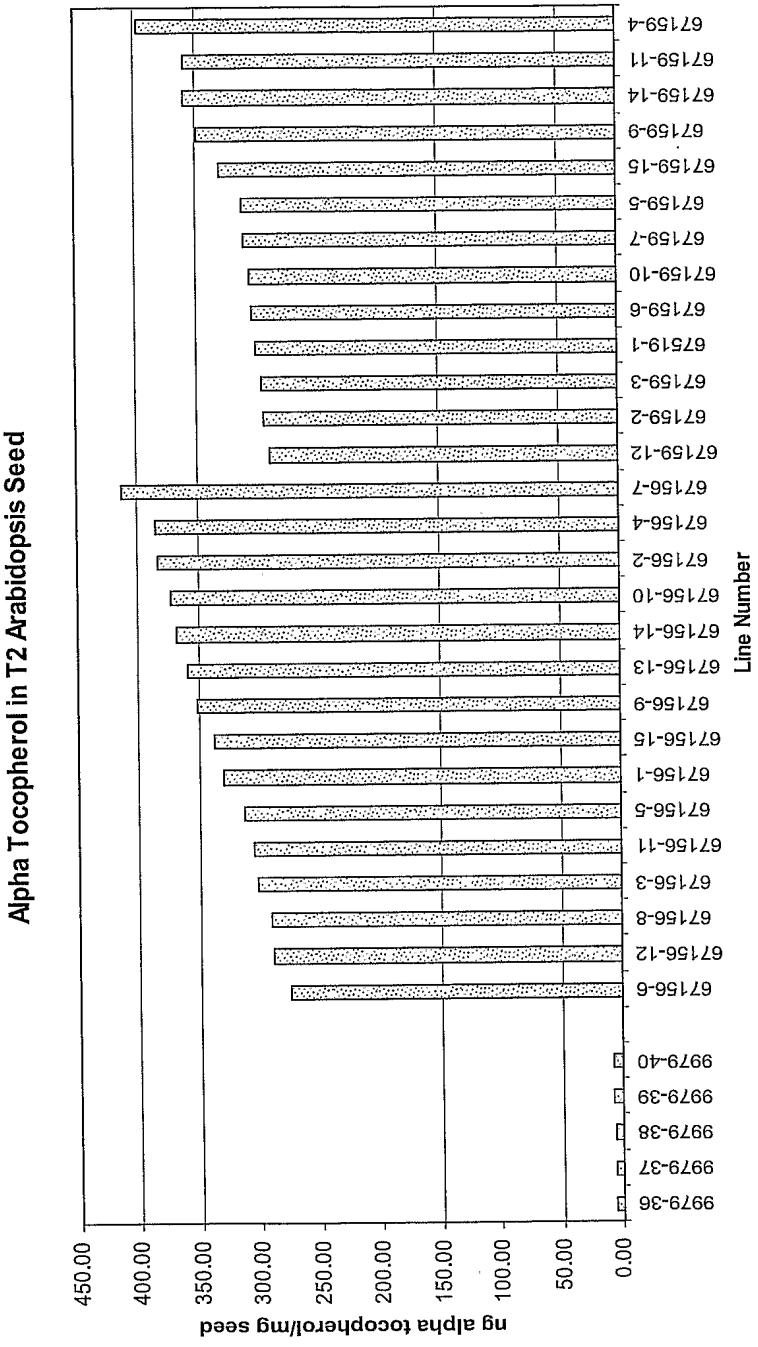
Alpha Tocopherol in T2 Arabidopsis Seed
ng alpha tocopherol/mg seed
450.00
400.00
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
Line Number
67159-4
67159-11
67159-14
67159-9
67159-15
67159-5
67159-7
67159-10
67159-6
67519-1
67159-3
67159-2
67159-12
67156-7
67156-4
67156-2
67156-10
67156-14
67156-13
67156-9
67156-15
67156-1
67156-5
67156-11
67156-3
67156-8
67156-12
67156-6
9979-40
9979-39
9979-38
9979-37
9979-36

FIG. 21A

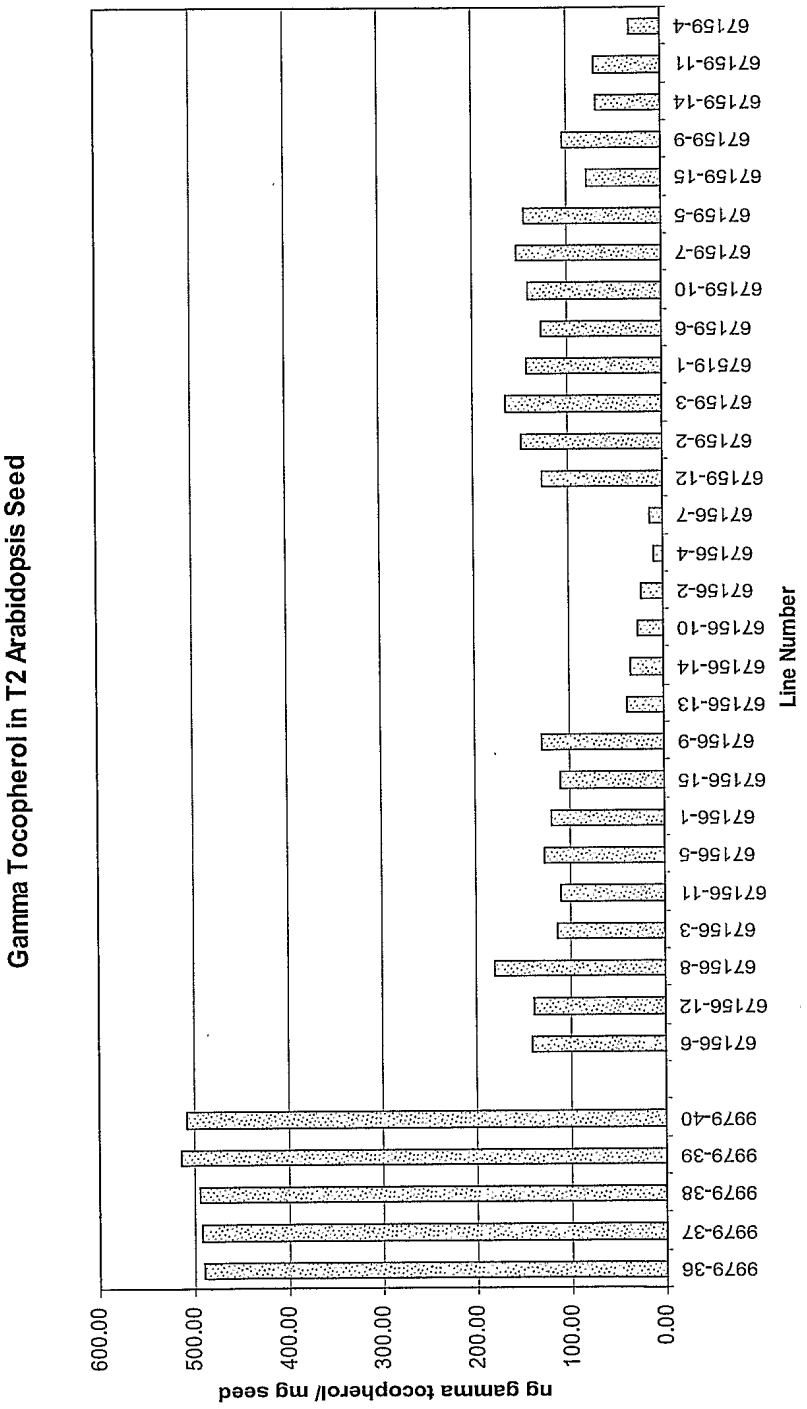
Gamma Tocopherol in T2 Arabidopsis Seed
ng gamma tocopherol/ mg seed
600.00
500.00
400.00
300.00
200.00
100.00
0.00
Line Number
9979-36
9979-37
9979-38
9979-39
9979-40
67156-6
67156-12
67156-8
67156-3
67156-11
67156-5
67156-1
67156-15
67156-9
67156-13
67156-14
67156-10
67156-2
67156-4
67156-7
67159-12
67159-2
67159-3
67519-1
67159-6
67159-10
67159-7
67159-5
67159-15
67159-9
67159-14
67159-11
67159-4

FIG. 21B

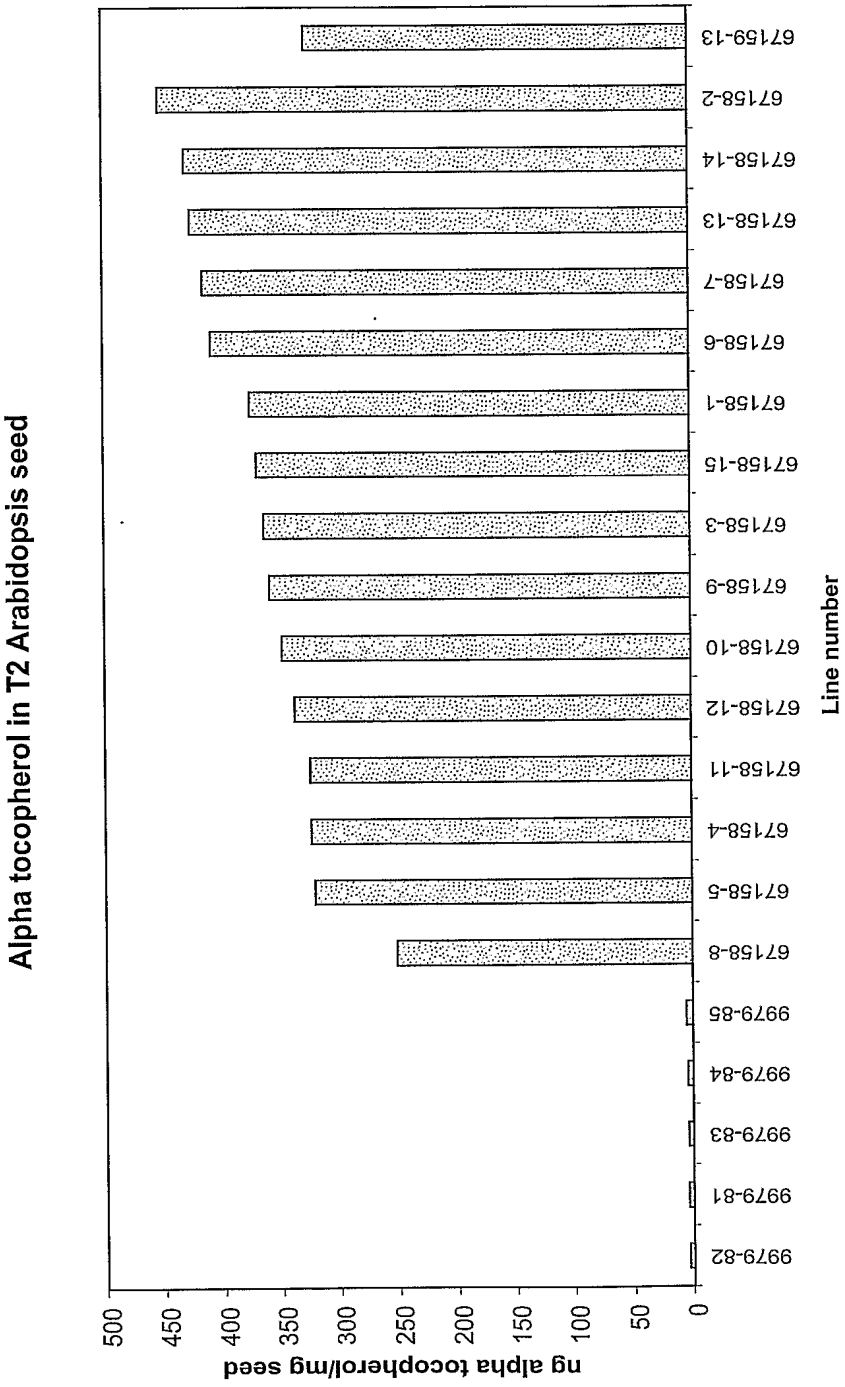
Alpha tocopherol in T2 Arabidopsis seed
ng alpha tocopherol/mg seed
0
50
100
150
200
250
300
350
400
450
500
Line number
9979-82
9979-81
9979-83
9979-84
9979-85
67158-8
67158-5
67158-4
67158-11
67158-12
67158-10
67158-9
67158-3
67158-15
67158-1
67158-6
67158-7
67158-13
67158-14
67158-2
67159-13

FIG. 22A

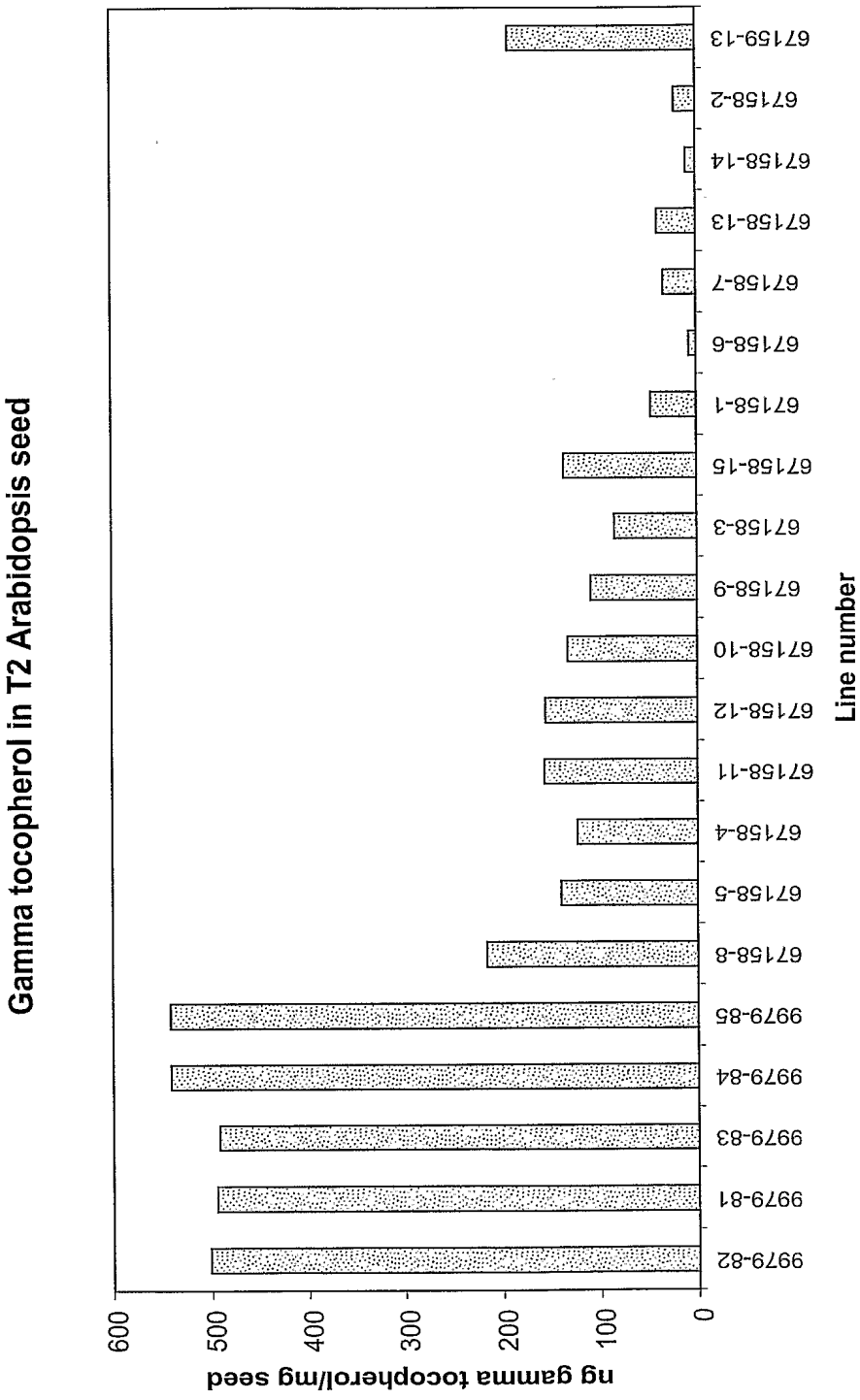
Gamma tocopherol in T2 Arabidopsis seed
ng gamma tocopherol/mg seed
0
100
200
300
400
500
600
Line number
9979-82
9979-81
9979-83
9979-84
9979-85
67158-8
67158-5
67158-4
67158-11
67158-12
67158-10
67158-9
67158-3
67158-15
67158-1
67158-6
67158-7
67158-13
67158-14
67158-2
67159-13

FIG. 22B

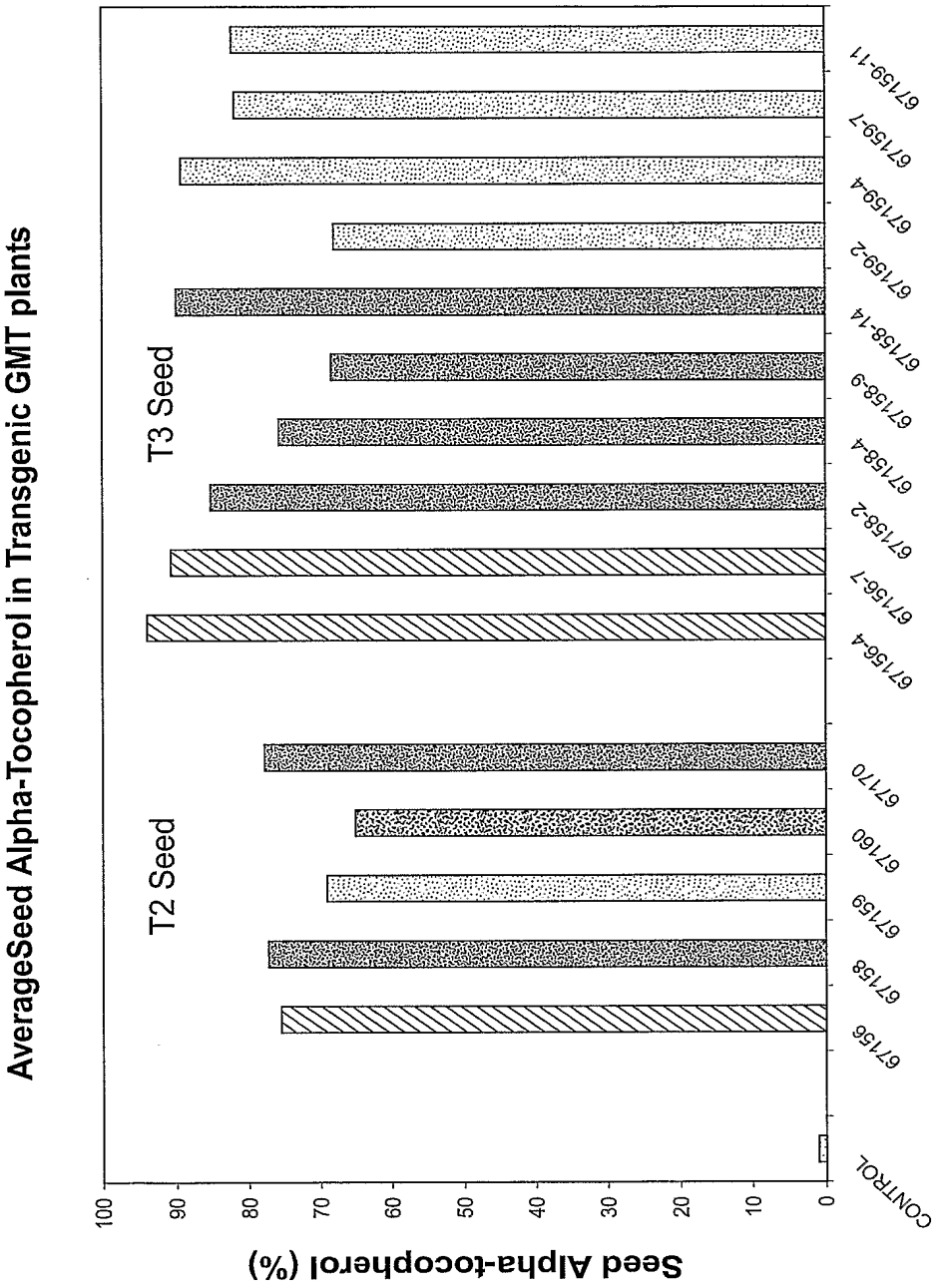
AverageSeed Alpha-Tocopherol in Transgenic GMT plants
Seed Alpha-tocopherol (%)
100
90
80
70
60
50
40
30
20
10
0
T2 Seed
T3 Seed
CONTROL
67156
67158
67159
67160
67170
67156-4
67156-7
67158-2
67158-4
67158-9
67158-14
67159-2
67159-4
67159-7
67159-11

FIG. 23

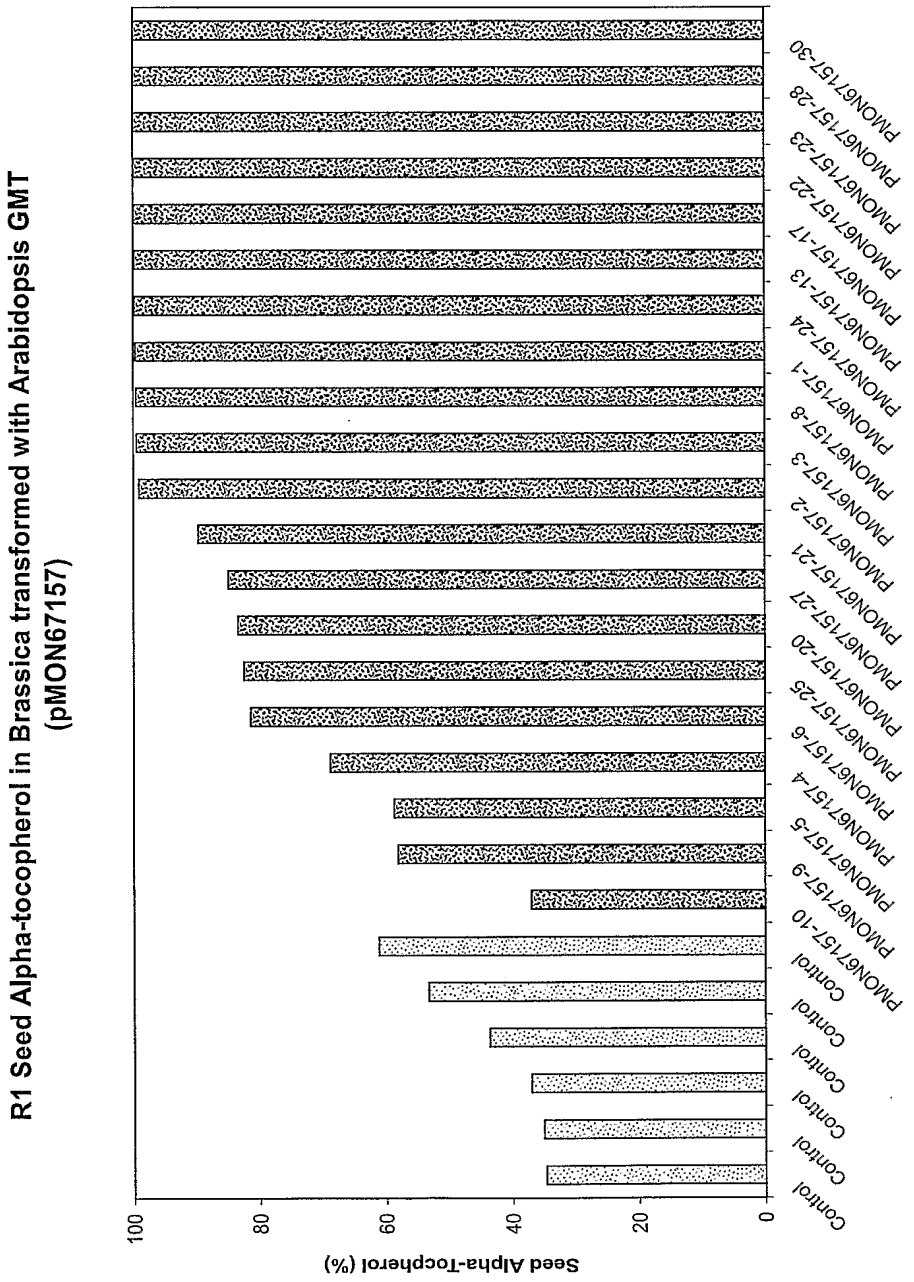
R1 Seed Alpha-tocopherol in Brassica transformed with Arabidopsis GMT
(pMON67157)
Seed Alpha-Tocopherol (%)
0
20
40
60
80
100
Control
Control
Control
Control
Control
Control
Control
PMON67157-10
PMON67157-9
PMON67157-5
PMON67157-4
PMON67157-6
PMON67157-25
PMON67157-20
PMON67157-27
PMON67157-21
PMON67157-2
PMON67157-3
PMON67157-8
PMON67157-1
PMON67157-24
PMON67157-13
PMON67157-17
PMON67157-22
PMON67157-23
PMON67157-28
PMON67157-30

FIG. 24

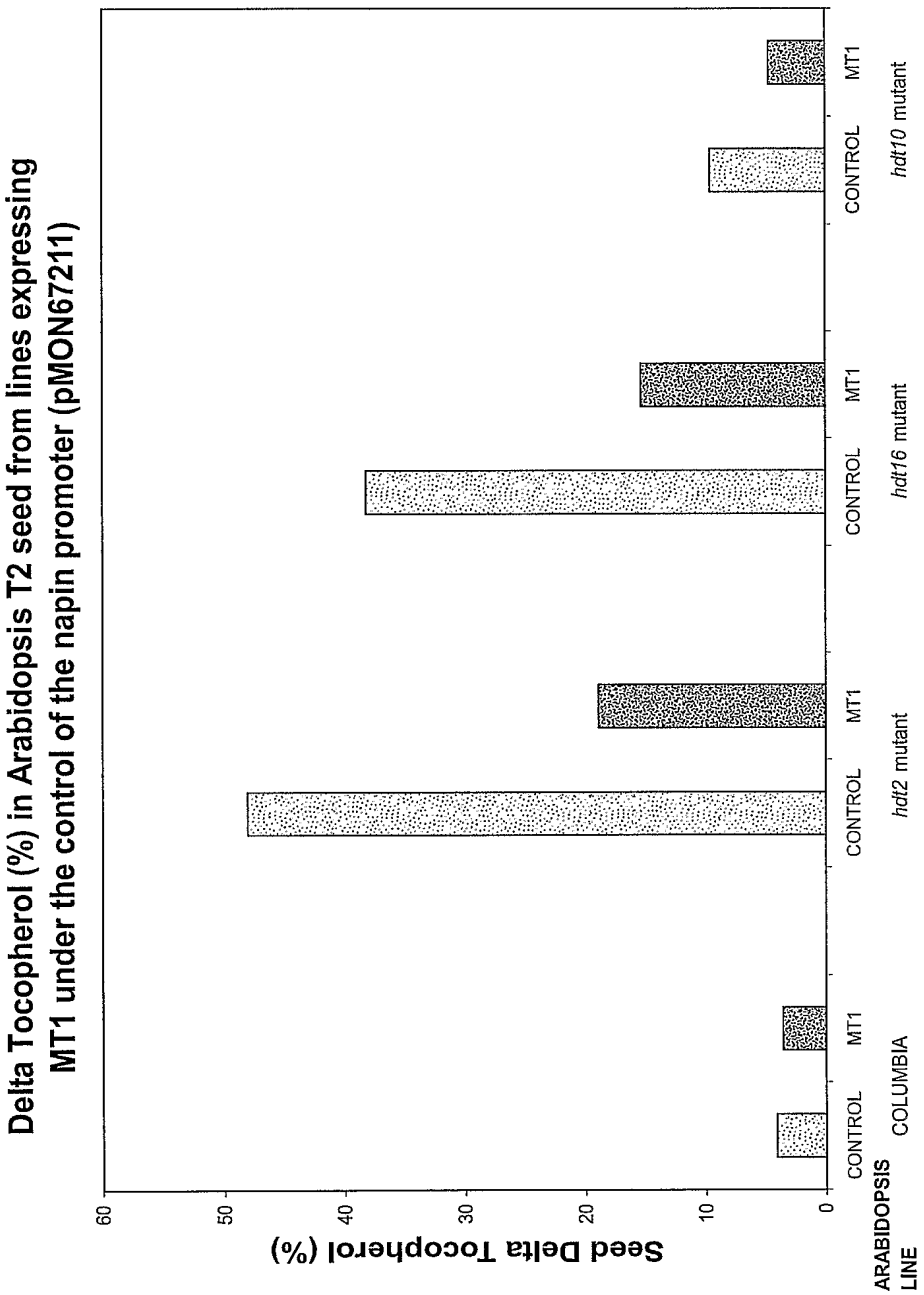
Delta Tocopherol (%) in Arabidopsis T2 seed from lines expressing
MT1 under the control of the napin promoter (pMON67211)
Seed Delta Tocopherol (%)
0
10
20
30
40
50
60
CONTROL
MT1
CONTROL
MT1
CONTROL
MT1
CONTROL
MT1
ARABIDOPSIS LINE
COLUMBIA
hdt2 mutant
hdt16 mutant
hdt10 mutant

FIG. 25

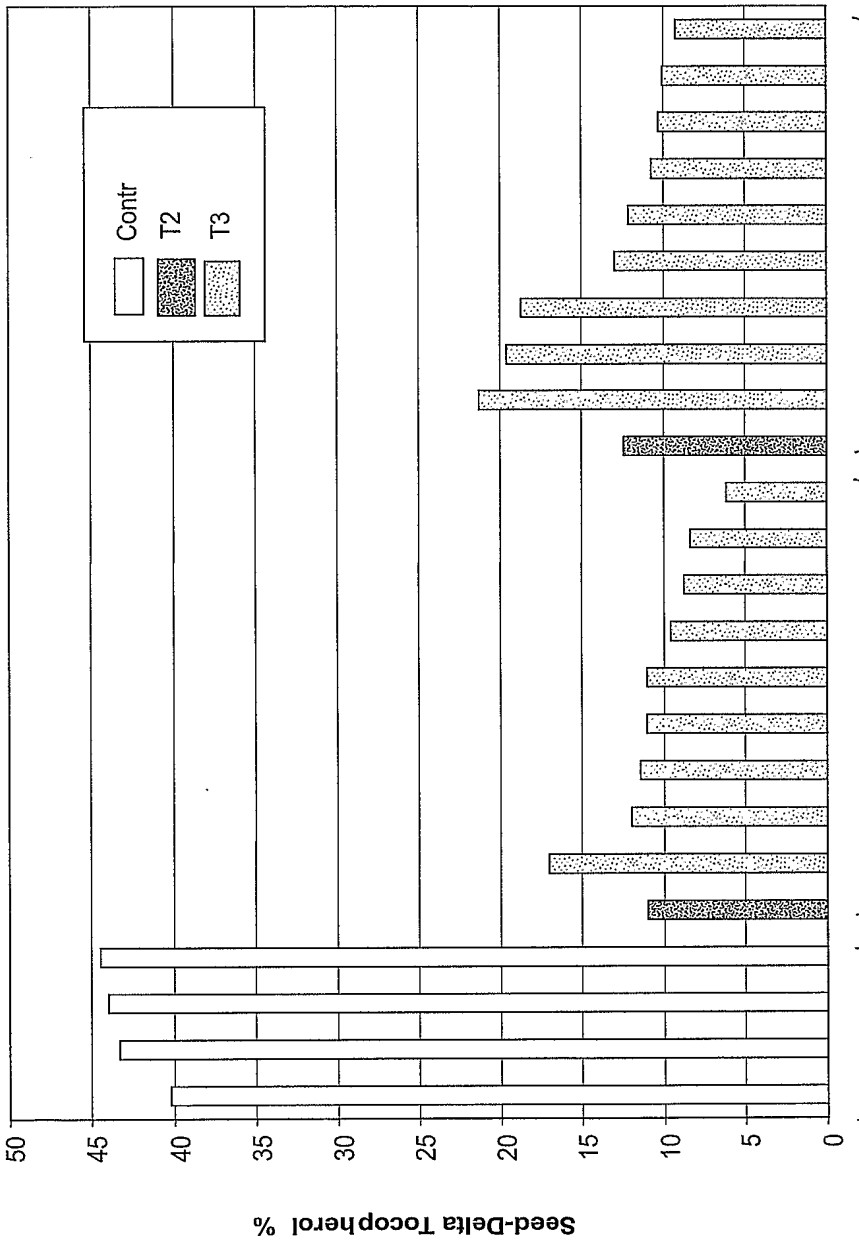
Contr
T2
T3
Seed-Delta Tocopherol %
50
45
40
35
30
25
20
15
10
5
0
hdt2
mutant
Line 6
Line 12

FIG. 26

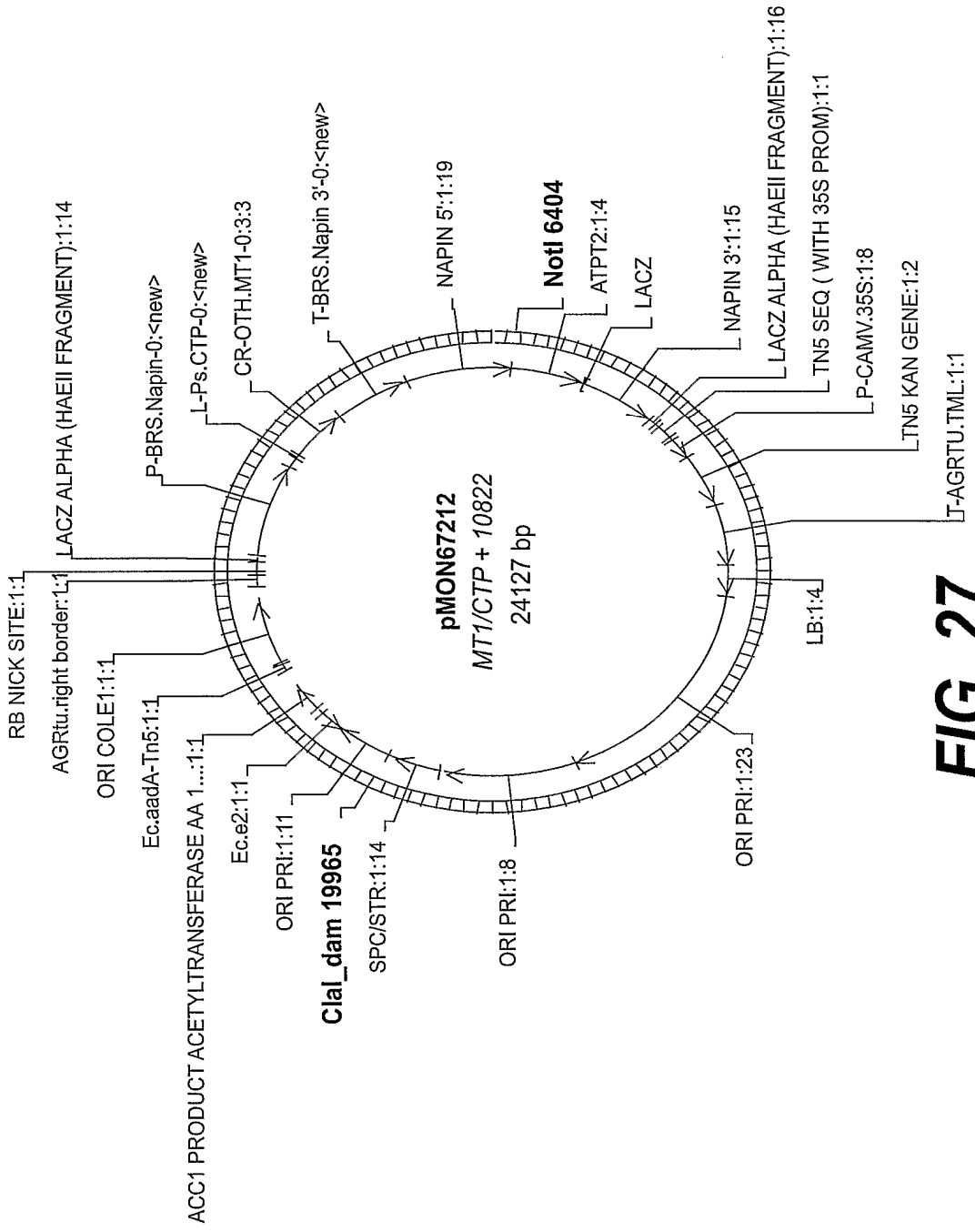
pMON67212
MT1/CTP + 10822
24127 bp
NotI 6404
ClaI_dam 19965
RB NICK SITE:1:1
AGRtu.right border:1:1
LACZ ALPHA (HAEII FRAGMENT):1:14
ORI COLE1:1:1
Ec.aadA-Tn5:1:1
ACC1 PRODUCT ACETYLTRANSFERASE AA 1...:1:1
Ec.e2:1:1
ORI PRI:1:11
SPC/STR:1:14
ORI PRI:1:8
ORI PRI:1:23
LB:1:4
T-AGRTU.TML:1:1
TN5 KAN GENE:1:2
P-CAMV.35S:1:8
TN5 SEQ (WITH 35S PROM):1:1
LACZ ALPHA (HAEII FRAGMENT):1:16
NAPIN 3':1:15
LACZ
ATPT2:1:4
NAPIN 5':1:19
T-BRS.Napin 3'-0:<new>
CR-OTH.MT1-0:3:3
L-Ps.CTP-0:<new>
P-BRS.Napin-0:<new>

FIG. 27

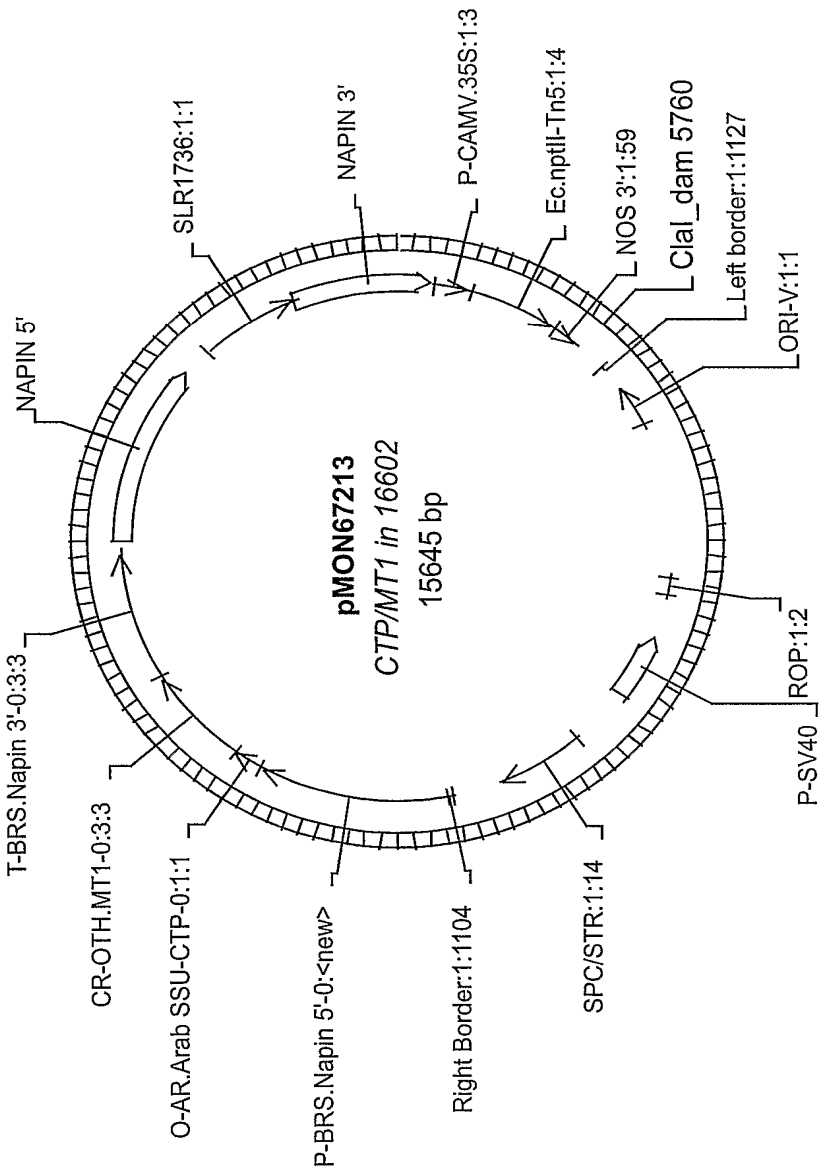
pMON67213
CTP/MT1 in 16602
15645 bp
NAPIN 5'
SLR1736:1:1
NAPIN 3'
P-CAMV.35S:1:3
Ec.nptII-Tn5:1:4
NOS 3':1:59
ClaI_dam 5760
Left border:1:1127
ORI-V:1:1
ROP:1:2
P-SV40
SPC/STR:1:14
Right Border:1:1104
P-BRS.Napin 5'-0:<new>
O-AR.Arab SSU-CTP-0:1:1
CR-OTH.MT1-0:3:3
T-BRS.Napin 3'-0:3:3

FIG. 28

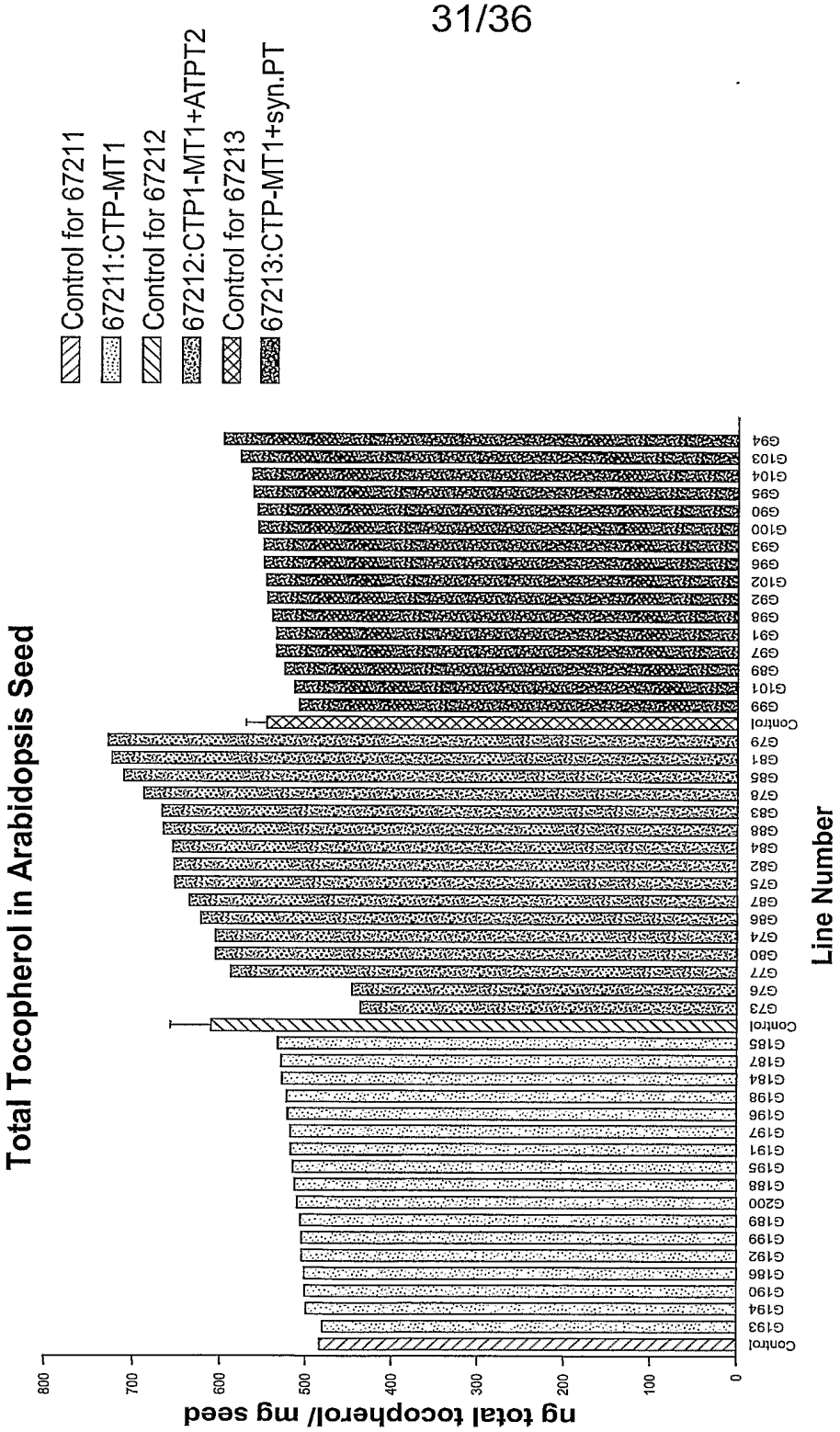
Total Tocopherol in Arabidopsis Seed
Control for 67211
67211:CTP-MT1
Control for 67212
67212:CTP1-MT1+ATPT2
Control for 67213
67213:CTP-MT1+syn.PT
ng total tocopherol/ mg seed
0
100
200
300
400
500
600
700
800
Line Number
Control
G193
G194
G190
G186
G192
G199
G189
G200
G188
G195
G191
G197
G196
G198
G184
G187
G185
Control
G73
G76
G77
G80
G74
G86
G87
G75
G82
G84
G88
G83
G78
G85
G81
G79
Control
G99
G101
G89
G97
G91
G98
G92
G102
G96
G93
G100
G90
G95
G104
G103
G94

FIG. 29

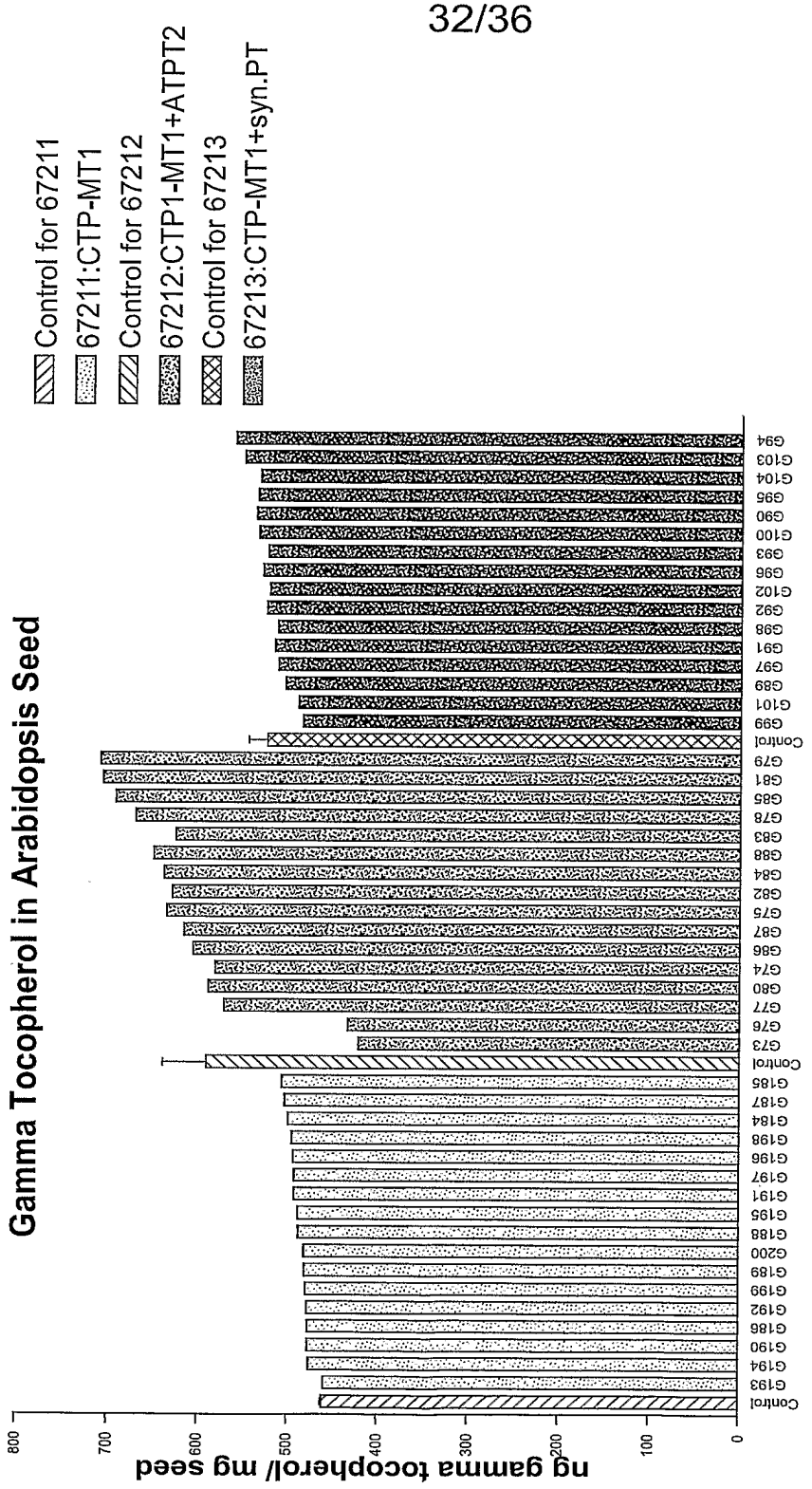
Gamma Tocopherol in Arabidopsis Seed
Control for 67211
67211:CTP-MT1
Control for 67212
67212:CTP1-MT1+ATPT2
Control for 67213
67213:CTP-MT1+syn.PT
ng gamma tocopherol/ mg seed
0
100
200
300
400
500
600
700
800
Line Number
Control
G193
G194
G190
G186
G192
G199
G189
G200
G188
G195
G191
G197
G196
G198
G184
G187
G185
Control
G73
G76
G77
G80
G74
G86
G87
G75
G82
G84
G88
G83
G78
G85
G81
G79
Control
G99
G101
G89
G97
G91
G98
G92
G102
G96
G93
G100
G90
G95
G104
G103
G94

FIG. 30

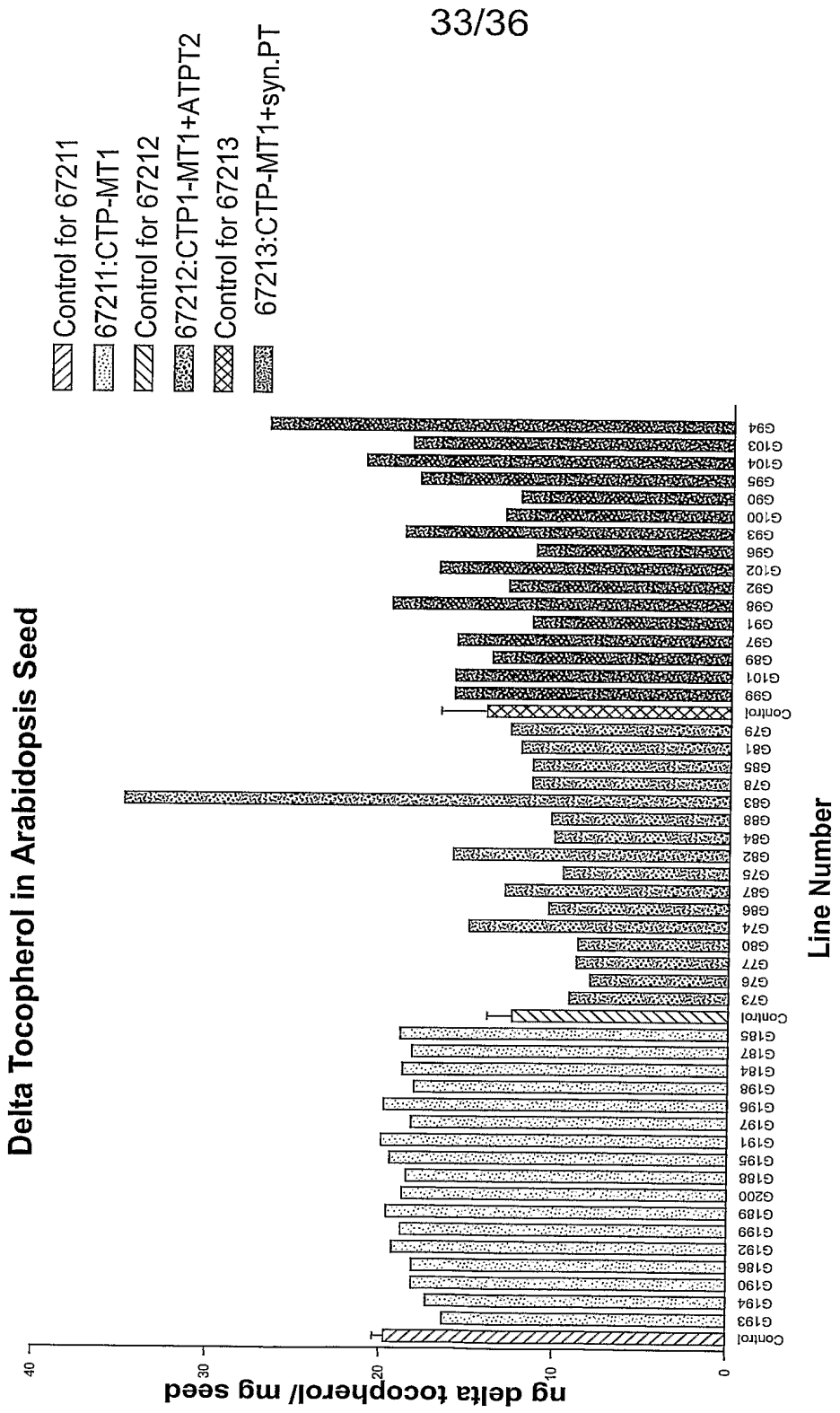
Delta Tocopherol in Arabidopsis Seed
Control for 67211
67211:CTP-MT1
Control for 67212
67212:CTP1-MT1+ATPT2
Control for 67213
67213:CTP-MT1+syn.PT
ng delta tocopherol/ mg seed
0
10
20
30
40
Line Number
Control
G193
G194
G190
G186
G192
G199
G189
G200
G188
G195
G191
G197
G196
G198
G184
G187
G185
Control
G73
G76
G77
G80
G74
G86
G87
G75
G82
G84
G88
G83
G78
G85
G81
G79
Control
G99
G101
G89
G97
G91
G98
G92
G102
G96
G93
G100
G90
G95
G104
G103
G94

FIG. 31

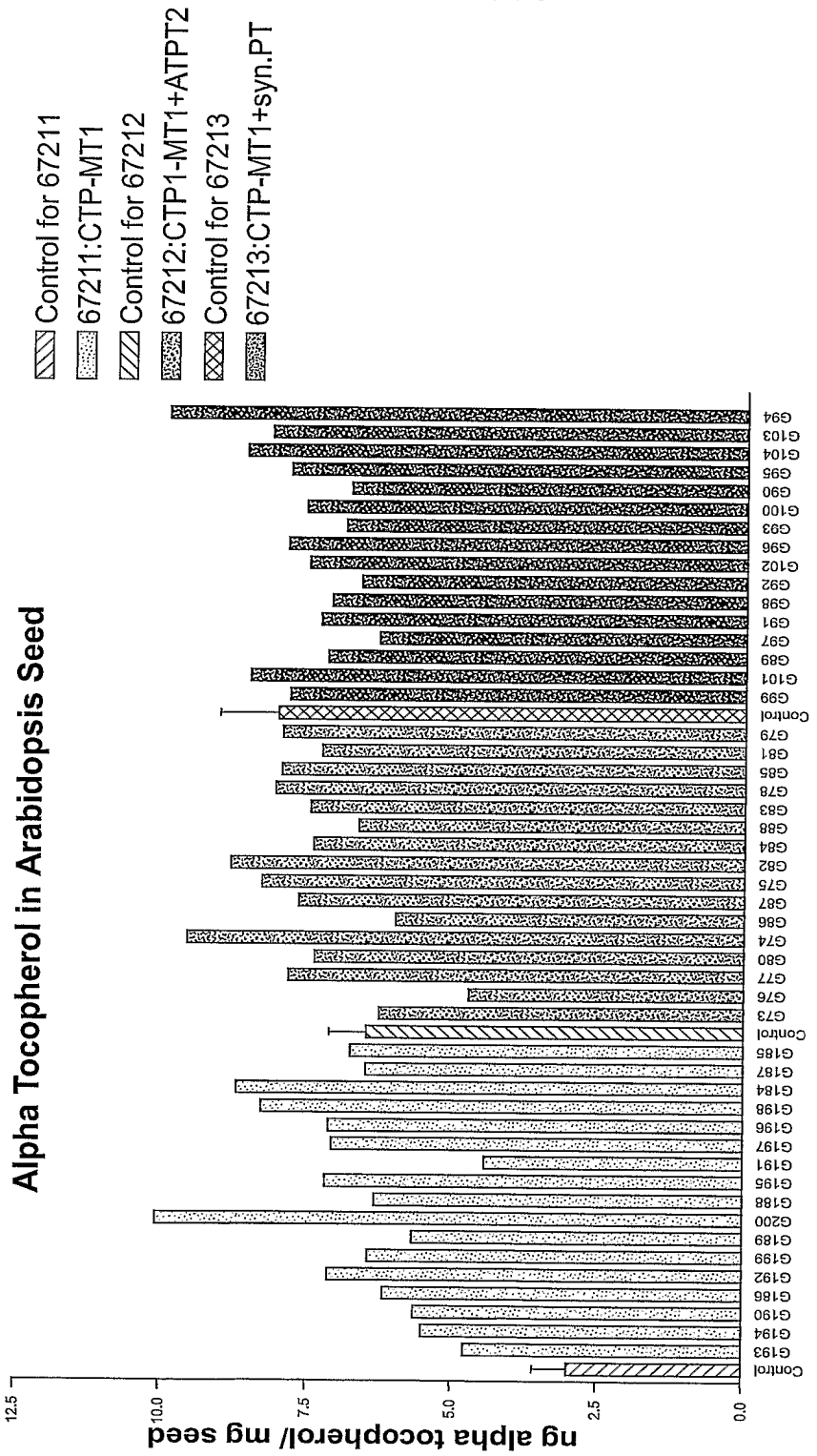
Alpha Tocopherol in Arabidopsis Seed
Control for 67211
67211:CTP-MT1
Control for 67212
67212:CTP1-MT1+ATPT2
Control for 67213
67213:CTP-MT1+syn.PT
ng alpha tocopherol/ mg seed
12.5
10.0
7.5
5.0
2.5
0.0
Line Number
Control
G193
G194
G190
G186
G192
G199
G189
G200
G188
G195
G191
G197
G196
G198
G184
G187
G185
Control
G73
G76
G77
G80
G74
G86
G87
G75
G82
G84
G88
G83
G78
G85
G81
G79
Control
G99
G101
G89
G97
G91
G98
G92
G102
G96
G93
G100
G90
G95
G104
G103
G94

FIG. 32

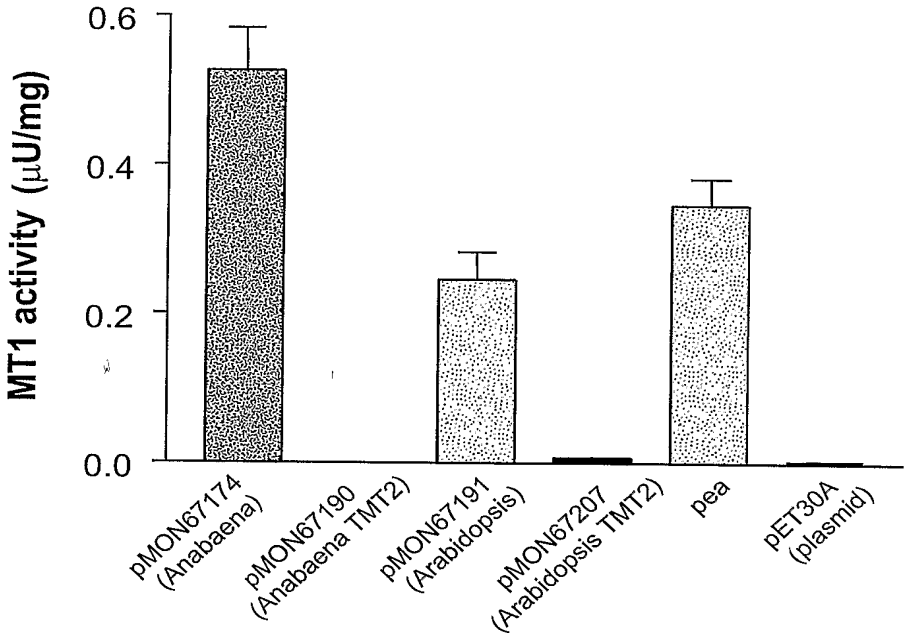
MT1 activity (μU/mg)
0.6
0.4
0.2
0.0
pMON67174 (Anabaena)
pMON67190 (Anabaena TMT2)
pMON67191 (Arabidopsis)
pMON67207 (Arabidopsis TMT2)
pea
pET30A (plasmid)

FIG. 33

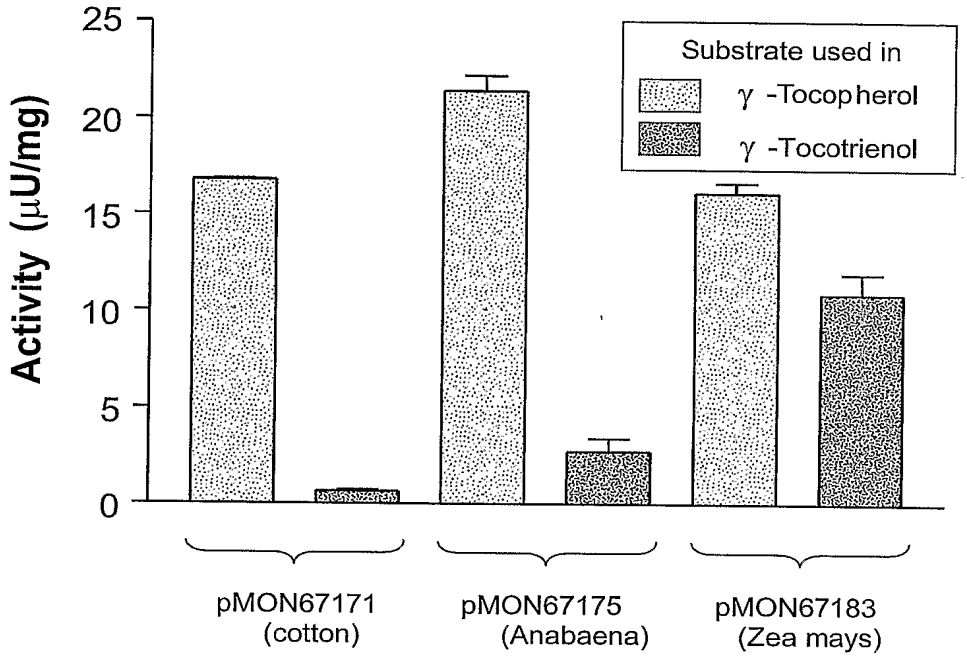
25
20
15
10
5
0
Activity (μU/mg)
Substrate used in
γ -Tocopherol
γ -Tocotrienol
pMON67171
(cotton)
pMON67175
(Anabaena)
pMON67183
(Zea mays)

FIG. 34

SEQUENCE LISTING
<110> Valentin, Henry
Hao, Ming
Van Eenennaam, Alison
Karunanandaa, Balasulojini
Aasen, Eric
Levering, Charlene

<120> METHYLTRANSFERASE GENES AND USES THEREOF

<130> 16515.156

<150> 60/312,758
<151> 2001-08-17

<160> 85

<210> 1
<211> 1047
<212> DNA
<213> Arabidopsis thaliana

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tctttcggct caaagtcatc gcttctcttt cggtctccat cctcctcctc ctcagtctct 120

atgacgacaa cgcgtggaaa cgtggctgtg gcggctgctg ctacatccac tgaggcgcta 180

agaaaaggaa tagcggagtt ctacaatgaa acttcgggtt tgtgggaaga gatttgggga 240

gatcatatgc atcatggctt ttatgaccct gattcttctg ttcaactttc tgattctggt 300

cacaaggaag ctcagatccg tatgattgaa gagtctctcc gtttcgccgg tgttactgat 360

gaagaggagg agaaaaagat aaagaaagta gtggatgttg ggtgtgggat tggaggaagc 420

tcaagatatc ttgcctctaa atttggagct gaatgcattg gcattactct cagccctgtt 480

caggccaaga gagccaatga tctcgcggct gctcaatcac tctctcataa ggcttccttc 540

caagttgcgg atgcgttgga tcagccattc gaagatggaa aattcgatct agtgtggtcg 600

atggagagtg gtgagcatat gcctgacaag gccaagtttg taaaagagtt ggtacgtgtg 660

gcggctccag gaggtaggat aataatagtg acatggtgcc atagaaatct atctgcgggg 720

gaggaagctt tgcagccgtg ggagcaaaac atcttggaca aaatctgtaa gacgttctat 780

ctcccggctt ggtgctccac cgatgattat gtcaacttgc ttcaatccca ttctctccag 840

gatattaagt gtgcggattg gtcagagaac gtagctcctt tctggcctgc ggttatacgg 900

actgcattaa catggaaggg ccttgtgtct ctgcttcgta gtggtatgaa aagtattaaa 960

ggagcattga caatgccatt gatgattgaa ggttacaaga aaggtgtcat taagtttggt 1020

atcatcactt gccagaagcc actctaa 1047

<210> 2
<211> 1047
<212> DNA
<213> Arabidopsis thaliana

<400> 2
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tctttcggct caaagtcatc gcttctcttt cggtctccat cctcctcctc ctcagtctct 120

atgacgacaa cgcgtggaaa cgtggctgtg gcggctgctg ctacatccac tgaggcgcta 180

agaaaaggaa tagcggagtt ctacaatgaa acttcgggtt tgtgggaaga gatttgggga 240

gatcatatgc atcatggctt ttatgaccct gattcttctg ttcaactttc tgattctggt 300

cacaaggaag ctcagatccg tatgattgaa gagtctctcc gttttgccgg tgttactgat 360

gaagaggagg agaaaaagat aaagaaagta gtggatgttg ggtgtgggat tggaggaagc 420

tcaagatatc ttgcctctaa atttggagct gaatgcattg gcattactct cagccctgtt 480

caggccaaga gagccaatga tctcgcggct gctcaatcac tcgctcataa ggcttccttc 540

caagttgcgg atgcgttgga tcagccattc gaagatggaa aattcgatct agtgtggtcg 600

atggagagtg gtgagcatat gcctgacaag gccaagtttg taaaagagtt ggtacgtgtg 660

gcggctccag gaggtaggat aataatagtg acatggtgcc atagaaatct atctgcgggg 720

gaggaagctt tgcagccgtg ggagcaaaac atcttggaca aaatctgtaa gacgttctat 780

ctcccggctt ggtgctccac cgatgattat gtcaacttgc ttcaatccca ttctctccag 840

gatattaagt gtgcggattg gtcagagaac gtagctcctt tctggcctgc ggttatacgg 900

actgcattaa catggaaggg ccttgtgtct ctgcttcgta gtggtatgaa aagtattaaa 960

ggagcattga caatgccatt gatgattgaa ggttacaaga aaggtgtcat taagtttggt 1020

atcatcactt gccagaagcc actctaa 1047

<210> 3
<211> 1095
<212> DNA
<213> Oryza sativa

<400> 3
atggcccacg ccgccgcggc cacgggcgca ctggcaccgc tgcatccact gctccgctgc 60

acgagccgtc atctctgcgc ctcggcttcc cctcgcgccg gcctctgcct ccaccaccac 120

cgccgccgcc gccgcagcag ccggaggacg aaactcgccg tgcgcgcgat ggcaccgacg 180

ttgtcctcgt cgtcgacggc ggcggcagct cccccggggc tgaaggaggg catcgcgggg 240

ctctacgacg agtcgtccgg cgtgtgggag agcatctggg gcgagcacat gcaccacggc 300

ttctacgacg ccggcgaggc cgcctccatg tccgaccacc gccgcgccca gatccgcatg 360

atcgaggaat ccctcgcctt cgccgccgtc cccggtgcag atgatgcgga gaagaaaccc 420

aaaagtgtag ttgatgttgg ctgtggcatt ggtggtagct caagatactt ggcgaacaaa 480

tacggagcgc aatgctacgg catcacgttg agtccggtgc aggctgaaag aggaaatgcc 540

ctcgcggcag agcaagggtt atcagacaag gtgcgtattc aagttggtga tgcattggag 600

cagccttttc ctgatgggca gtttgatctt gtctggtcca tggagagtgg cgagcacatg 660

ccagacaaac ggcagtttgt aagcgagctg gcacgcgtcg cagctcctgg ggcgagaata 720

atcattgtga cctggtgcca taggaacctc gagccatccg aagagtccct gaaacctgat 780

gagctgaatc tcctgaaaag gatatgcgat gcatattatc tcccagactg gtgctctcct 840

tctgattatg tcaaaattgc cgagtcactg tctcttgagg atataaggac agctgattgg 900

tcagagaacg tcgccccatt ctggcctgcg gttataaaat cagcattgac atggaaaggt 960

ttaacttctc tgctaagaag tgggtggaag acgataagag gtgcaatggt gatgcctctg 1020

atgatcgaag gatacaagaa agggctcatc aaattcacca tcatcacctg tcgcaagccc 1080

gaaacaacgc agtag 1095

<210> 4
<211> 1038
<212> DNA
<213> Gossypium hirsutum

<400> 4
atggctgccg cgttacaatt acaaacacac ccttgcttcc atggcacgtg ccaactctca 60

cctccgccac gaccttccgt ttccttcccc tcttcctccc gctcgtttcc atctagcaga 120

cgttccctgt ccgcgcatgt gaaggcggcg gcgtcgtctt tgtccaccac caccttgcag 180

gaagggatag cggagtttta cgatgagtcg tcggggattt gggaagacat atggggtgac 240

catatgcacc atggatatta cgagccgggt tccgatattt cgggttcaga tcatcgtgcc 300

gctcagattc gaatggtcga agaatcgctc cgttttgctg gaatatcaga ggacccagca 360

aacaggccca agagaatagt tgatgttggg tgtgggatag gaggcagttc taggtatcta 420

gcaaggaaat atggggcaaa atgccaaggc attactttga gccctgttca agctggaaga 480

gccaatgctc ttgctaatgc tcaaggacta gcagaacagg tttgttttga agttgcagat 540

gccttgaacc aaccattccc tgatgaccaa tttgatcttg tttggtctat ggaaagcgga 600

gaacacatgc ctgacaaacc caagtttgtt aaagagctgg tgcgagtggc agctccagga 660

ggcacaataa tagtagtgac atggtgccat agggatcttg gtccatctga agagtctttg 720

cagccatggg agcaaaagct tttaaacaga atatgtgatg cttactattt accagagtgg 780

tgttctactt ctgattatgt caaattattt cagtccctat ctctccagga tataaaggca 840

ggagactgga ctgagaatgt agcacccttt tggccagcag tgatacgttc agcattgaca 900

tggaagggct tcacatcgct gctacgaagt ggattaaaaa caataaaagg tgcactggtg 960

atgccattga tgatcgaagg tttccagaaa ggggtgataa agtttgccat cattgcttgc 1020

cggaagccag ctgagtag 1038

<210> 5
<211> 1131
<212> DNA
<213> Cuphea pulcherrima

<400> 5
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agctccaaga acatggcacc gcccgaactg gctcagtcgc aagtacctat gggaagtaac 120

aagagcaaca agaaccacgg cttggtcggt tcggtttctg gttggagaag gatgtttggg 180

acatgggcta ctgccgacaa gactcagagt accgatacgt ctaatgaagg cgtggttagt 240

tacgatactc aggtcttgca gaagggtata gcggagttct atgacgagtc gtcgggtata 300

tgggaggata tatggggaga tcacatgcat catggctact atgatggttc cactcctgtc 360

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tctggtatgg tccatggaga gtggcgagca catgccggac aagagaaagt ttgttagtga 660

gctggcacgc gtcgctgctc ctggagggac aataatcatc gtgacatggt gccataggaa 720

cctcgaacca tctgagactt cgctaaaacc cgatgaactg agtctcttga agaggatttg 780

cgatgcgtac tacctcccag actggtgctc accttcagac tatgtgaaca tcgccaaatc 840

actgtctctg gaggatatca aggcagctga ttggtcagag aatgtggccc cattttggcc 900

cgctgtgata aaatcagcac taacatggaa gggcctcacc tctctactga caagcggatg 960

gaagacgatc agaggggcga tggtgatgcc gctgatgatc caaggttaca agaaggggct 1020

catcaaattc accatcatca cctgtcgcaa gcctggagca gcgtaggtga ccaaggggca 1080

gaagttactg tcaaagcacc tctgctaagt ccaataatgt agatccatgg ccccatcacc 1140

gtctattgta ctgtactgta ctgtaccaga atgaacagtc tcctgggaca tgttttccaa 1200

ttgccatgac atgtcaaatg atcttctacc 1230

<210> 16
<211> 843
<212> DNA
<213> Nostoc punctiforme

<400> 16
atgagtgcaa cactttacca gcaaattcag caattttacg atgcttcatc tggtctgtgg 60

gaacagatat ggggcgaaca catgcaccac ggctattacg gcgctgatgg tacccagaaa 120

aaagaccgcc gtcaggctca aattgattta atcgaagaat tgcttaattg ggcaggggta 180

caagcagcag aagatatact agatgtgggt tgtggaattg gcggtagttc tttatacctg 240

gcgcaaaagt ttaatgctaa agctacaggg attacattga gtcctgtaca agctgcaaga 300

gcaacagaac gcgcattgga agctaatttg agtctgagaa cacagttcca agtcgctaat 360

gctcaagcaa tgcccttttgc tgacgattct tttgacttgg tttggtcgct ggaaagtggc 420

gaacacatgc cagataaaac caagtttctt caggagtgct atcgagtact gaagcctggt 480

ggcaagttaa ttatggtgac ttggtgtcat cgaccaactg atgaatctcc attaacggca 540

gatgaggaaa agcacttgca ggatatttat cgggtgtatt gtttgcctta tgtgatttct 600

ttgccagagt atgaagcgat cgcacatcaa ctaccattac ataatatccg cactgctgat 660

tggtcaactg ctgtcgcccc cttttggaat gtggtaattg attctgcatt cactccccaa 720

gcgctttggg gtttactaaa tgctggttgg actaccattc aaggggcatt atcactggga 780

ttaatgcgtc gcggttatga acgtgggtta attcggtttg gcttactgtg cggcaataag 840

tag 843

<210> 17
<211> 843
<212> DNA
<213> Anabaena sp.

<400> 17
atgagtgcaa cactttacca acaaattcag caattttacg atgcttcctc tgggctgtgg 60

gaagagattt ggggcgaaca tatgcaccac ggctattatg gtgcagacgg tactgaacaa 120

aaaaaccgcc gtcaggcgca aattgattta attgaagaat tactcacttg ggcaggagta 180

caaacagcag aaaatatact agatgtgggt tgtggtattg gtggtagttc tctgtatttg 240

gcaggaaagt tgaatgctaa agctacagga attaccctga gtccagtgca agccgctaga 300

gccacagaaa gagccaagga agctggttta agtggtagaa gtcagttttt agtggcaaat 360

gcccaagcaa tgccttttga tgataattct tttgacttgg tgtggtcgct agaaagtggc 420

gaacatatgc cagataaaac caagtttttg caagagtgtt atcgagtctt gaaaccgggc 480

ggtaagttaa tcatggtgac atggtgtcat cgtcccactg ataaaacacc actgacggct 540

gatgaaaaaa aacacctaga agatatttat cgggtgtatt gtttgcctta tgtaatttcg 600

ttgccggagt atgaagcgat cgcacgtcaa ctaccattaa ataatatccg caccgccgac 660

tggtcgcaat ccgtcgccca attttggaac atagtcatcg attccgcctt tacccccaa 720

gcaatattcg gcttactccg cgcaggttgg actaccatcc aaggagcctt atcactaggc 780

ttaatgcgtc gcggctatga gcgcgggtta attcggtttg ggttgctttg tggggataag 840

tga 843

<210> 18
<211> 348
<212> PRT
<213> Arabidopsis thaliana

<400> 18

Met Lys Ala Thr Leu Ala Ala Pro Ser Ser Leu Thr Ser Leu Pro Tyr
1 5 10 15

Arg Thr Asn Ser Ser Phe Gly Ser Lys Ser Ser Leu Leu Phe Arg Ser
20 25 30

Pro Ser Ser Ser Ser Ser Val Ser Met Thr Thr Thr Arg Gly Asn Val
35 40 45

Ala Val Ala Ala Ala Ala Thr Ser Thr Glu Ala Leu Arg Lys Gly Ile
50 55 60

Ala Glu Phe Tyr Asn Glu Thr Ser Gly Leu Trp Glu Glu Ile Trp Gly
65 70 75 80

Asp His Met His His Gly Phe Tyr Asp Pro Asp Ser Ser Val Gln Leu
85 90 95

Ser Asp Ser Gly His Lys Glu Ala Gln Ile Arg Met Ile Glu Glu Ser
100 105 110

Leu Arg Phe Ala Gly Val Thr Asp Glu Glu Glu Glu Lys Lys Ile Lys
115 120 125

Lys Val Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu
130 135 140

Ala Ser Lys Phe Gly Ala Glu Cys Ile Gly Ile Thr Leu Ser Pro Val
145 150 155 160

Gln Ala Lys Arg Ala Asn Asp Leu Ala Ala Ala Gln Ser Leu Ser His
165 170 175

Lys Ala Ser Phe Gln Val Ala Asp Ala Leu Asp Gln Pro Phe Glu Asp
180 185 190

Gly Lys Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro
195 200 205

Asp Lys Ala Lys Phe Val Lys Glu Leu Val Arg Val Ala Ala Pro Gly
210 215 220

Gly Arg Ile Ile Ile Val Thr Trp Cys His Arg Asn Leu Ser Ala Gly
225 230 235 240

Glu Glu Ala Leu Gln Pro Trp Glu Gln Asn Ile Leu Asp Lys Ile Cys
245 250 255

Lys Thr Phe Tyr Leu Pro Ala Trp Cys Ser Thr Asp Asp Tyr Val Asn
260 265 270

Leu Leu Gln Ser His Ser Leu Gln Asp Ile Lys Cys Ala Asp Trp Ser
275 280 285

Glu Asn Val Ala Pro Phe Trp Pro Ala Val Ile Arg Thr Ala Leu Thr
290 295 300

Trp Lys Gly Leu Val Ser Leu Leu Arg Ser Gly Met Lys Ser Ile Lys
305 310 315 320

Gly Ala Leu Thr Met Pro Leu Met Ile Glu Gly Tyr Lys Lys Gly Val
325 330 335

Ile Lys Phe Gly Ile Ile Thr Cys Gln Lys Pro Leu
340 345

<210> 19
<211> 348
<212> PRT
<213> Arabidopsis thaliana

<400> 19
Met Lys Ala Thr Leu Ala Ala Pro Ser Ser Leu Thr Ser Leu Pro Tyr
1 5 10 15

Arg Thr Asn Ser Ser Phe Gly Ser Lys Ser Ser Leu Leu Phe Arg Ser
20 25 30

Pro Ser Ser Ser Ser Ser Val Ser Met Thr Thr Thr Arg Gly Asn Val
35 40 45

Ala Val Ala Ala Ala Ala Thr Ser Thr Glu Ala Leu Arg Lys Gly Ile
50 55 60

```
Ala Glu Phe Tyr Asn Glu Thr Ser Gly Leu Trp Glu Glu Ile Trp Gly
65                  70                  75                  80

Asp His Met His His Gly Phe Tyr Asp Pro Asp Ser Ser Val Gln Leu
                85                  90                  95

Ser Asp Ser Gly His Lys Glu Ala Gln Ile Arg Met Ile Glu Glu Ser
            100                 105                 110

Leu Arg Phe Ala Gly Val Thr Asp Glu Glu Glu Glu Lys Lys Ile Lys
        115                 120                 125

Lys Val Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu
    130                 135                 140

Ala Ser Lys Phe Gly Ala Glu Cys Ile Gly Ile Thr Leu Ser Pro Val
145                 150                 155                 160

Gln Ala Lys Arg Ala Asn Asp Leu Ala Ala Ala Gln Ser Leu Ala His
                165                 170                 175

Lys Ala Ser Phe Gln Val Ala Asp Ala Leu Asp Gln Pro Phe Glu Asp
            180                 185                 190

Gly Lys Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro
        195                 200                 205

Asp Lys Ala Lys Phe Val Lys Glu Leu Val Arg Val Ala Ala Pro Gly
    210                 215                 220

Gly Arg Ile Ile Ile Val Thr Trp Cys His Arg Asn Leu Ser Ala Gly
225                 230                 235                 240

Glu Glu Ala Leu Gln Pro Trp Glu Gln Asn Ile Leu Asp Lys Ile Cys
                245                 250                 255

Lys Thr Phe Tyr Leu Pro Ala Trp Cys Ser Thr Asp Asp Tyr Val Asn
            260                 265                 270

Leu Leu Gln Ser His Ser Leu Gln Asp Ile Lys Cys Ala Asp Trp Ser
        275                 280                 285

Glu Asn Val Ala Pro Phe Trp Pro Ala Val Ile Arg Thr Ala Leu Thr
    290                 295                 300

Trp Lys Gly Leu Val Ser Leu Leu Arg Ser Gly Met Lys Ser Ile Lys
305                 310                 315                 320
```

```
Gly Ala Leu Thr Met Pro Leu Met Ile Glu Gly Tyr Lys Lys Gly Val
                325                 330                 335

Ile Lys Phe Gly Ile Ile Thr Cys Gln Lys Pro Leu
            340                 345
```

<210> 20
<211> 364
<212> PRT
<213> Oryza sativa

<400> 20

```
Met Ala His Ala Ala Ala Ala Thr Gly Ala Leu Ala Pro Leu His Pro
1               5                   10                  15

Leu Leu Arg Cys Thr Ser Arg His Leu Cys Ala Ser Ala Ser Pro Arg
            20                  25                  30

Ala Gly Leu Cys Leu His His His Arg Arg Arg Arg Arg Ser Ser Arg
        35                  40                  45

Arg Thr Lys Leu Ala Val Arg Ala Met Ala Pro Thr Leu Ser Ser Ser
    50                  55                  60

Ser Thr Ala Ala Ala Ala Pro Pro Gly Leu Lys Glu Gly Ile Ala Gly
65                  70                  75                  80

Leu Tyr Asp Glu Ser Ser Gly Val Trp Glu Ser Ile Trp Gly Glu His
                85                  90                  95

Met His His Gly Phe Tyr Asp Ala Gly Glu Ala Ala Ser Met Ser Asp
            100                 105                 110

His Arg Arg Ala Gln Ile Arg Met Ile Glu Glu Ser Leu Ala Phe Ala
        115                 120                 125

Ala Val Pro Gly Ala Asp Asp Ala Glu Lys Lys Pro Lys Ser Val Val
    130                 135                 140

Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu Ala Asn Lys
145                 150                 155                 160

Tyr Gly Ala Gln Cys Tyr Gly Ile Thr Leu Ser Pro Val Gln Ala Glu
                165                 170                 175

Arg Gly Asn Ala Leu Ala Ala Glu Gln Gly Leu Ser Asp Lys Val Arg
            180                 185                 190

Ile Gln Val Gly Asp Ala Leu Glu Gln Pro Phe Pro Asp Gly Gln Phe
        195                 200                 205
```

```
Asp Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro Asp Lys Arg
    210                 215                 220

Gln Phe Val Ser Glu Leu Ala Arg Val Ala Ala Pro Gly Ala Arg Ile
225                 230                 235                 240

Ile Ile Val Thr Trp Cys His Arg Asn Leu Glu Pro Ser Glu Glu Ser
                245                 250                 255

Leu Lys Pro Asp Glu Leu Asn Leu Leu Lys Arg Ile Cys Asp Ala Tyr
            260                 265                 270

Tyr Leu Pro Asp Trp Cys Ser Pro Ser Asp Tyr Val Lys Ile Ala Glu
        275                 280                 285

Ser Leu Ser Leu Glu Asp Ile Arg Thr Ala Asp Trp Ser Glu Asn Val
    290                 295                 300

Ala Pro Phe Trp Pro Ala Val Ile Lys Ser Ala Leu Thr Trp Lys Gly
305                 310                 315                 320

Leu Thr Ser Leu Leu Arg Ser Gly Trp Lys Thr Ile Arg Gly Ala Met
                325                 330                 335

Val Met Pro Leu Met Ile Glu Gly Tyr Lys Lys Gly Leu Ile Lys Phe
            340                 345                 350

Thr Ile Ile Thr Cys Arg Lys Pro Glu Thr Thr Gln
        355                 360
```

<210> 21
<211> 352
<212> PRT
<213> Zea mays

```
<400> 21
Met Ala His Ala Ala Leu Leu His Cys Ser Gln Ser Ser Arg Ser Leu
1               5                   10                  15

Ala Ala Cys Arg Arg Gly Ser His Tyr Arg Ala Pro Ser His Val Pro
            20                  25                  30

Arg His Ser Arg Arg Leu Arg Arg Ala Val Val Ser Leu Arg Pro Met
        35                  40                  45

Ala Ser Ser Thr Ala Gln Ala Pro Ala Thr Ala Pro Pro Gly Leu Lys
    50                  55                  60
```

```
Glu Gly Ile Ala Gly Leu Tyr Asp Glu Ser Ser Gly Leu Trp Glu Asn
65                  70                  75                  80

Ile Trp Gly Asp His Met His His Gly Phe Tyr Asp Ser Ser Glu Ala
                85                  90                  95

Ala Ser Met Ala Asp His Arg Arg Ala Gln Ile Arg Met Ile Glu Glu
            100                 105                 110

Ala Leu Ala Phe Ala Gly Val Pro Ala Ser Asp Asp Pro Glu Lys Thr
        115                 120                 125

Pro Lys Thr Ile Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg
    130                 135                 140

Tyr Leu Ala Lys Lys Tyr Gly Ala Gln Cys Thr Gly Ile Thr Leu Ser
145                 150                 155                 160

Pro Val Gln Ala Glu Arg Gly Asn Ala Leu Ala Ala Ala Gln Gly Leu
                165                 170                 175

Ser Asp Gln Val Thr Leu Gln Val Ala Asp Ala Leu Glu Gln Pro Phe
            180                 185                 190

Pro Asp Gly Gln Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His
        195                 200                 205

Met Pro Asp Lys Arg Lys Phe Val Ser Glu Leu Ala Arg Val Ala Ala
    210                 215                 220

Pro Gly Gly Thr Ile Ile Ile Val Thr Trp Cys His Arg Asn Leu Asp
225                 230                 235                 240

Pro Ser Glu Thr Ser Leu Lys Pro Asp Glu Leu Ser Leu Leu Arg Arg
                245                 250                 255

Ile Cys Asp Ala Tyr Tyr Leu Pro Asp Trp Cys Ser Pro Ser Asp Tyr
            260                 265                 270

Val Asn Ile Ala Lys Ser Leu Ser Leu Glu Asp Ile Lys Thr Ala Asp
        275                 280                 285

Trp Ser Glu Asn Val Ala Pro Phe Trp Pro Ala Val Ile Lys Ser Ala
    290                 295                 300

Leu Thr Trp Lys Gly Phe Thr Ser Leu Leu Thr Thr Gly Trp Lys Thr
305                 310                 315                 320
```

Ile Arg Gly Ala Met Val Met Pro Leu Met Ile Gln Gly Tyr Lys Lys
325 330 335

Gly Leu Ile Lys Phe Thr Ile Ile Thr Cys Arg Lys Pro Gly Ala Ala
340 345 350

<210> 22
<211> 345
<212> PRT
<213> Gossypium hirsutum

<400> 22
Met Ala Ala Ala Leu Gln Leu Gln Thr His Pro Cys Phe His Gly Thr
1 5 10 15

Cys Gln Leu Ser Pro Pro Pro Arg Pro Ser Val Ser Phe Pro Ser Ser
20 25 30

Ser Arg Ser Phe Pro Ser Ser Arg Arg Ser Leu Ser Ala His Val Lys
35 40 45

Ala Ala Ala Ser Ser Leu Ser Thr Thr Thr Leu Gln Glu Gly Ile Ala
50 55 60

Glu Phe Tyr Asp Glu Ser Ser Gly Ile Trp Glu Asp Ile Trp Gly Asp
65 70 75 80

His Met His His Gly Tyr Tyr Glu Pro Gly Ser Asp Ile Ser Gly Ser
85 90 95

Asp His Arg Ala Ala Gln Ile Arg Met Val Glu Glu Ser Leu Arg Phe
100 105 110

Ala Gly Ile Ser Glu Asp Pro Ala Asn Arg Pro Lys Arg Ile Val Asp
115 120 125

Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu Ala Arg Lys Tyr
130 135 140

Gly Ala Lys Cys Gln Gly Ile Thr Leu Ser Pro Val Gln Ala Gly Arg
145 150 155 160

Ala Asn Ala Leu Ala Asn Ala Gln Gly Leu Ala Glu Gln Val Cys Phe
165 170 175

Glu Val Ala Asp Ala Leu Asn Gln Pro Phe Pro Asp Asp Gln Phe Asp
180 185 190

Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro Asp Lys Pro Lys
195 200 205

```
Phe Val Lys Glu Leu Val Arg Val Ala Ala Pro Gly Gly Thr Ile Ile
    210                 215                 220

Val Val Thr Trp Cys His Arg Asp Leu Gly Pro Ser Glu Glu Ser Leu
225                 230                 235                 240

Gln Pro Trp Glu Gln Lys Leu Leu Asn Arg Ile Cys Asp Ala Tyr Tyr
                245                 250                 255

Leu Pro Glu Trp Cys Ser Thr Ser Asp Tyr Val Lys Leu Phe Gln Ser
            260                 265                 270

Leu Ser Leu Gln Asp Ile Lys Ala Gly Asp Trp Thr Glu Asn Val Ala
        275                 280                 285

Pro Phe Trp Pro Ala Val Ile Arg Ser Ala Leu Thr Trp Lys Gly Phe
    290                 295                 300

Thr Ser Leu Leu Arg Ser Gly Leu Lys Thr Ile Lys Gly Ala Leu Val
305                 310                 315                 320

Met Pro Leu Met Ile Glu Gly Phe Gln Lys Gly Val Ile Lys Phe Ala
                325                 330                 335

Ile Ile Ala Cys Arg Lys Pro Ala Glu
            340                 345
```

<210> 23
<211> 376
<212> PRT
<213> Cuphea pulcherrima

```
<400> 23
Met Pro Ile Thr Ser Ile Ser Ala Asn Gln Arg Pro Phe Phe Pro Ser
1               5                   10                  15

Pro Tyr Arg Gly Ser Ser Lys Asn Met Ala Pro Pro Glu Leu Ala Gln
            20                  25                  30

Ser Gln Val Pro Met Gly Ser Asn Lys Ser Asn Lys Asn His Gly Leu
        35                  40                  45

Val Gly Ser Val Ser Gly Trp Arg Arg Met Phe Gly Thr Trp Ala Thr
    50                  55                  60

Ala Asp Lys Thr Gln Ser Thr Asp Thr Ser Asn Glu Gly Val Val Ser
65                  70                  75                  80
```

```
Tyr Asp Thr Gln Val Leu Gln Lys Gly Ile Ala Glu Phe Tyr Asp Glu
                85                  90                  95

Ser Ser Gly Ile Trp Glu Asp Ile Trp Gly Asp His Met His His Gly
            100                 105                 110

Tyr Tyr Asp Gly Ser Thr Pro Val Ser Leu Pro Asp His Arg Ser Ala
        115                 120                 125

Gln Ile Arg Met Ile Asp Glu Ala Leu Arg Phe Ala Ser Val Pro Ser
    130                 135                 140

Gly Glu Glu Asp Glu Ser Lys Ser Lys Ile Pro Lys Arg Ile Val Asp
145                 150                 155                 160

Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu Ala Arg Lys Tyr
                165                 170                 175

Gly Ala Glu Cys Arg Gly Ile Thr Leu Ser Pro Val Gln Ala Glu Arg
            180                 185                 190

Gly Asn Ser Leu Ala Arg Ser Gln Gly Leu Ser Asp Lys Val Ser Phe
        195                 200                 205

Gln Val Ala Asp Ala Leu Ala Gln Pro Phe Pro Asp Gly Gln Phe Asp
    210                 215                 220

Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro Asp Lys Ser Lys
225                 230                 235                 240

Phe Val Asn Glu Leu Val Arg Val Ala Ala Pro Gly Gly Thr Ile Ile
                245                 250                 255

Ile Val Thr Trp Cys His Arg Asp Leu Arg Glu Asp Glu Asp Ala Leu
            260                 265                 270

Gln Pro Arg Glu Lys Glu Ile Leu Asp Lys Ile Cys Asn Pro Phe Tyr
        275                 280                 285

Leu Pro Ala Trp Cys Ser Ala Ala Asp Tyr Val Lys Leu Leu Gln Ser
    290                 295                 300

Leu Asp Val Glu Asp Ile Lys Ser Ala Asp Trp Thr Pro Tyr Val Ala
305                 310                 315                 320

Pro Phe Trp Pro Ala Val Leu Lys Ser Ala Phe Thr Ile Lys Gly Phe
                325                 330                 335
```

Val Ser Leu Leu Arg Ser Gly Met Lys Thr Ile Lys Gly Ala Phe Ala
340 345 350

Met Pro Leu Met Ile Glu Gly Tyr Lys Lys Gly Val Ile Lys Phe Ser
355 360 365

Ile Ile Thr Cys Arg Lys Pro Glu
370 375

<210> 24
<211> 347
<212> PRT
<213> Brassica napus

<400> 24
Met Lys Ala Thr Leu Ala Pro Ser Ser Leu Ile Ser Leu Pro Arg His
1 5 10 15

Lys Val Ser Ser Leu Arg Ser Pro Ser Leu Leu Leu Gln Ser Gln Arg
20 25 30

Pro Ser Ser Ala Leu Met Thr Thr Thr Thr Ala Ser Arg Gly Ser Val
35 40 45

Ala Val Thr Ala Ala Ala Thr Ser Ser Val Glu Ala Leu Arg Glu Gly
50 55 60

Ile Ala Glu Phe Tyr Asn Glu Thr Ser Gly Leu Trp Glu Glu Ile Trp
65 70 75 80

Gly Asp His Met His His Gly Phe Tyr Asp Pro Asp Ser Ser Val Gln
85 90 95

Leu Ser Asp Ser Gly His Arg Glu Ala Gln Ile Arg Met Ile Glu Glu
100 105 110

Ser Leu Arg Phe Ala Gly Val Thr Glu Glu Glu Lys Lys Ile Lys Arg
115 120 125

Val Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Ile Ala
130 135 140

Ser Lys Phe Gly Ala Glu Cys Ile Gly Ile Thr Leu Ser Pro Val Gln
145 150 155 160

Ala Lys Arg Ala Asn Asp Leu Ala Ala Ala Gln Ser Leu Ser His Lys
165 170 175

Val Ser Phe Gln Val Ala Asp Ala Leu Glu Gln Pro Phe Glu Asp Gly
180 185 190

```
Ile Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro Asp
        195                 200                 205

Lys Ala Lys Phe Val Lys Glu Leu Val Arg Val Ala Ala Pro Gly Gly
    210                 215                 220

Arg Ile Ile Ile Val Thr Trp Cys His Arg Asn Leu Ser Pro Gly Glu
225                 230                 235                 240

Glu Ala Leu Gln Pro Trp Glu Gln Asn Leu Leu Asp Arg Ile Cys Lys
                245                 250                 255

Thr Phe Tyr Leu Pro Ala Trp Cys Ser Thr Ser Asp Tyr Val Asp Leu
            260                 265                 270

Leu Gln Ser Leu Ser Leu Gln Asp Ile Lys Cys Ala Asp Trp Ser Glu
        275                 280                 285

Asn Val Ala Pro Phe Trp Pro Ala Val Ile Arg Thr Ala Leu Thr Trp
    290                 295                 300

Lys Gly Leu Val Ser Leu Leu Arg Ser Gly Met Lys Ser Ile Lys Gly
305                 310                 315                 320

Ala Leu Thr Met Pro Leu Met Ile Glu Gly Tyr Lys Lys Gly Val Ile
                325                 330                 335

Lys Phe Gly Ile Ile Thr Cys Gln Lys Pro Leu
            340                 345
```

<210> 25
<211> 347
<212> PRT
<213> Brassica napus

```
<400> 25
Met Lys Ala Thr Leu Ala Pro Pro Ser Ser Leu Ile Ser Leu Pro Arg
1               5                   10                  15

His Lys Val Ser Ser Leu Arg Ser Pro Ser Leu Leu Leu Gln Ser Gln
            20                  25                  30

Arg Arg Ser Ser Ala Leu Met Thr Thr Thr Ala Ser Arg Gly Ser Val
        35                  40                  45

Ala Val Thr Ala Ala Ala Thr Ser Ser Ala Glu Ala Leu Arg Glu Gly
    50                  55                  60
```

```
Ile Ala Glu Phe Tyr Asn Glu Thr Ser Gly Leu Trp Glu Glu Ile Trp
65                  70                  75                  80

Gly Asp His Met His His Gly Phe Tyr Asp Pro Asp Ser Ser Val Gln
                85                  90                  95

Leu Ser Asp Ser Gly His Arg Glu Ala Gln Ile Arg Met Ile Glu Glu
            100                 105                 110

Ser Leu Arg Phe Ala Gly Val Thr Glu Glu Glu Lys Lys Ile Lys Arg
        115                 120                 125

Val Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Ile Ala
    130                 135                 140

Ser Lys Phe Gly Ala Glu Cys Ile Gly Ile Thr Leu Ser Pro Val Gln
145                 150                 155                 160

Ala Lys Arg Ala Asn Asp Leu Ala Thr Ala Gln Ser Leu Ser His Lys
                165                 170                 175

Val Ser Phe Gln Val Ala Asp Ala Leu Asp Gln Pro Phe Glu Asp Gly
            180                 185                 190

Ile Ser Asp Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro Asp
        195                 200                 205

Lys Ala Lys Phe Val Lys Glu Leu Val Arg Val Thr Ala Pro Gly Gly
    210                 215                 220

Arg Ile Ile Ile Val Thr Trp Cys His Arg Asn Leu Ser Gln Gly Glu
225                 230                 235                 240

Glu Ser Leu Gln Pro Trp Glu Gln Asn Leu Leu Asp Arg Ile Cys Lys
                245                 250                 255

Thr Phe Tyr Leu Pro Ala Trp Cys Ser Thr Thr Asp Tyr Val Glu Leu
            260                 265                 270

Leu Gln Ser Leu Ser Leu Gln Asp Ile Lys Tyr Ala Asp Trp Ser Glu
        275                 280                 285

Asn Val Ala Pro Phe Trp Pro Ala Val Ile Arg Thr Ala Leu Thr Trp
    290                 295                 300

Lys Gly Leu Val Ser Leu Leu Arg Ser Gly Met Lys Ser Ile Lys Gly
305                 310                 315                 320
```

```
Ala Leu Thr Met Pro Leu Met Ile Glu Gly Tyr Lys Lys Gly Val Ile
                325                 330                 335

Lys Phe Gly Ile Ile Thr Cys Gln Lys Pro Leu
            340                 345
```

<210> 26
<211> 310
<212> PRT
<213> Lycopersicon esculentum

<400> 26

```
Met Ala Ser Val Ala Ala Met Asn Ala Val Ser Ser Ser Ser Val Glu
1               5                   10                  15

Val Gly Ile Gln Asn Gln Gln Glu Leu Lys Lys Gly Ile Ala Asp Leu
            20                  25                  30

Tyr Asp Glu Ser Ser Gly Ile Trp Glu Asp Ile Trp Gly Asp His Met
        35                  40                  45

His His Gly Tyr Tyr Glu Pro Lys Ser Ser Val Glu Leu Ser Asp His
    50                  55                  60

Arg Ala Ala Gln Ile Arg Met Ile Glu Gln Ala Leu Ser Phe Ala Ala
65                  70                  75                  80

Ile Ser Glu Asp Pro Ala Lys Lys Pro Thr Ser Ile Val Asp Val Gly
                85                  90                  95

Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu Ala Lys Lys Tyr Gly Ala
            100                 105                 110

Thr Ala Lys Gly Ile Thr Leu Ser Pro Val Gln Ala Glu Arg Ala Gln
        115                 120                 125

Ala Leu Ala Asp Ala Gln Gly Leu Gly Asp Lys Val Ser Phe Gln Val
    130                 135                 140

Ala Asp Ala Leu Asn Gln Pro Phe Pro Asp Gly Gln Phe Asp Leu Val
145                 150                 155                 160

Trp Ser Met Glu Ser Gly Glu His Met Pro Asn Lys Glu Lys Phe Val
                165                 170                 175

Gly Glu Leu Ala Arg Val Ala Ala Pro Gly Gly Thr Ile Ile Leu Val
            180                 185                 190

Thr Trp Cys His Arg Asp Leu Ser Pro Ser Glu Glu Ser Leu Thr Pro
        195                 200                 205
```

```
Glu Glu Lys Glu Leu Leu Asn Lys Ile Cys Lys Ala Phe Tyr Leu Pro
    210                 215                 220

Ala Trp Cys Ser Thr Ala Asp Tyr Val Lys Leu Leu Gln Ser Asn Ser
225                 230                 235                 240

Leu Gln Asp Ile Lys Ala Glu Asp Trp Ser Glu Asn Val Ala Pro Phe
                245                 250                 255

Trp Pro Ala Val Ile Lys Ser Ala Leu Thr Trp Lys Gly Phe Thr Ser
            260                 265                 270

Val Leu Arg Ser Gly Trp Lys Thr Ile Lys Ala Ala Leu Ala Met Pro
        275                 280                 285

Leu Met Ile Glu Gly Tyr Lys Lys Gly Leu Ile Lys Phe Ala Ile Ile
    290                 295                 300

Thr Cys Arg Lys Pro Glu
305                 310
```

<210> 27
<211> 302
<212> PRT
<213> Glycine max

<400> 27

```
Met Ser Val Glu Gln Lys Ala Ala Gly Lys Glu Glu Glu Gly Lys Leu
1               5                   10                  15

Gln Lys Gly Ile Ala Glu Phe Tyr Asp Glu Ser Ser Gly Ile Trp Glu
            20                  25                  30

Asn Ile Trp Gly Asp His Met His His Gly Phe Tyr Asp Pro Asp Ser
        35                  40                  45

Thr Val Ser Val Ser Asp His Arg Ala Ala Gln Ile Arg Met Ile Gln
    50                  55                  60

Glu Ser Leu Arg Phe Ala Ser Leu Leu Ser Glu Asn Pro Ser Lys Trp
65                  70                  75                  80

Pro Lys Ser Ile Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg
                85                  90                  95

Tyr Leu Ala Lys Lys Phe Gly Ala Thr Ser Val Gly Ile Thr Leu Ser
            100                 105                 110
```

```
Pro Val Gln Ala Gln Arg Ala Asn Ala Leu Ala Ala Ala Gln Gly Leu
        115                 120                 125

Ala Asp Lys Val Ser Phe Gln Val Ala Asp Ala Leu Gln Gln Pro Phe
    130                 135                 140

Ser Asp Gly Gln Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His
145                 150                 155                 160

Met Pro Asp Lys Ala Lys Phe Val Gly Glu Leu Ala Arg Val Ala Ala
                165                 170                 175

Pro Gly Ala Thr Ile Ile Ile Val Thr Trp Cys His Arg Asp Leu Gly
            180                 185                 190

Pro Asp Glu Gln Ser Leu His Pro Trp Glu Gln Asp Leu Leu Lys Lys
        195                 200                 205

Ile Cys Asp Ala Tyr Tyr Leu Pro Ala Trp Cys Ser Thr Ser Asp Tyr
    210                 215                 220

Val Lys Leu Leu Gln Ser Leu Ser Leu Gln Asp Ile Lys Ser Glu Asp
225                 230                 235                 240

Trp Ser Arg Phe Val Ala Pro Phe Trp Pro Ala Val Ile Arg Ser Ala
                245                 250                 255

Phe Thr Trp Lys Gly Leu Thr Ser Leu Leu Ser Ser Gly Gln Lys Thr
            260                 265                 270

Ile Lys Gly Ala Leu Ala Met Pro Leu Met Ile Glu Gly Tyr Lys Lys
        275                 280                 285

Asp Leu Ile Lys Phe Ala Ile Ile Thr Cys Arg Lys Pro Glu
    290                 295                 300
```

<210> 28
<211> 350
<212> PRT
<213> Glycine max

<400> 28

```
Met Ala Thr Val Val Arg Ile Pro Thr Ile Ser Cys Ile His Ile His
1               5                   10                  15

Thr Phe Arg Ser Gln Ser Pro Arg Thr Phe Ala Arg Ile Arg Val Gly
            20                  25                  30

Pro Arg Ser Trp Ala Pro Ile Arg Ala Ser Ala Ala Ser Ser Glu Arg
        35                  40                  45
```

Gly Glu Ile Val Leu Glu Gln Lys Pro Lys Lys Glu Glu Glu Gly Lys
50 55 60

Leu Gln Lys Gly Ile Ala Glu Phe Tyr Asp Glu Ser Ser Gly Leu Trp
65 70 75 80

Glu Asn Ile Trp Gly Asp His Met His His Gly Phe Tyr Asp Pro Asp
85 90 95

Ser Thr Val Ser Val Ser Asp His Arg Ala Ala Gln Ile Arg Met Ile
100 105 110

Gln Glu Ser Leu Arg Phe Ala Ser Val Ser Glu Glu Arg Ser Lys Trp
115 120 125

Pro Lys Ser Ile Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg
130 135 140

Tyr Leu Ala Lys Lys Phe Gly Ala Thr Ser Val Gly Ile Thr Leu Ser
145 150 155 160

Pro Val Gln Ala Gln Arg Ala Asn Ala Leu Ala Ala Ala Gln Gly Leu
165 170 175

Ala Asp Lys Val Ser Phe Gln Val Ala Asp Ala Leu Gln Gln Pro Phe
180 185 190

Ser Asp Gly Gln Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His
195 200 205

Met Pro Asp Lys Ala Lys Phe Val Gly Glu Leu Ala Arg Val Ala Ala
210 215 220

Pro Gly Ala Thr Ile Ile Ile Val Thr Trp Cys His Arg Asp Leu Gly
225 230 235 240

Pro Asp Glu Gln Ser Leu His Pro Trp Glu Gln Asp Leu Leu Lys Lys
245 250 255

Ile Cys Asp Ala Tyr Tyr Leu Pro Ala Trp Cys Ser Thr Ser Asp Tyr
260 265 270

Val Lys Leu Leu Gln Ser Leu Ser Leu Gln Asp Ile Lys Ser Glu Asp
275 280 285

Trp Ser Arg Phe Val Ala Pro Phe Trp Pro Ala Val Ile Arg Ser Ala
290 295 300

Phe Thr Trp Lys Gly Leu Thr Ser Leu Leu Ser Ser Gly Leu Lys Thr
305 310 315 320

Ile Lys Gly Ala Leu Ala Met Pro Leu Met Ile Glu Gly Tyr Lys Lys
325 330 335

Asp Leu Ile Lys Phe Ala Ile Ile Thr Cys Arg Lys Pro Glu
340 345 350

<210> 29
<211> 350
<212> PRT
<213> Glycine max

<400> 29
Met Ala Thr Val Val Arg Ile Pro Thr Ile Ser Cys Ile His Ile His
1 5 10 15

Thr Phe Arg Ser Gln Ser Pro Arg Thr Phe Ala Arg Ile Arg Val Gly
20 25 30

Pro Arg Ser Trp Ala Pro Ile Arg Ala Ser Ala Ala Ser Ser Glu Arg
35 40 45

Gly Glu Ile Val Leu Glu Gln Lys Pro Lys Lys Asp Asp Lys Glu Lys
50 55 60

Leu Gln Lys Gly Ile Ala Glu Phe Tyr Asp Glu Ser Ser Gly Leu Trp
65 70 75 80

Glu Asn Ile Trp Gly Asp His Met His His Gly Phe Tyr Asp Pro Asp
85 90 95

Ser Thr Val Ser Leu Ser Asp His Arg Ala Ala Gln Ile Arg Met Ile
100 105 110

Gln Glu Ser Leu Arg Phe Ala Ser Val Ser Glu Glu Arg Ser Lys Trp
115 120 125

Pro Lys Ser Ile Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg
130 135 140

Tyr Leu Ala Lys Lys Phe Gly Ala Thr Ser Val Gly Ile Thr Leu Ser
145 150 155 160

Pro Val Gln Ala Gln Arg Ala Asn Ala Leu Ala Ala Ala Gln Gly Leu
165 170 175

```
Ala Asp Lys Val Ser Phe Gln Val Ala Asp Ala Leu Gln Gln Pro Phe
            180                 185                 190

Ser Asp Gly Gln Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His
        195                 200                 205

Met Pro Asp Lys Ala Lys Phe Val Gly Glu Leu Ala Arg Val Ala Ala
    210                 215                 220

Pro Gly Ala Thr Ile Ile Ile Val Thr Trp Cys His Arg Asp Leu Gly
225                 230                 235                 240

Pro Asp Glu Gln Ser Leu His Pro Trp Glu Gln Asp Leu Leu Lys Lys
                245                 250                 255

Ile Cys Asp Ala Tyr Tyr Leu Pro Ala Trp Cys Ser Thr Ser Asp Tyr
            260                 265                 270

Val Lys Leu Leu Gln Ser Leu Ser Leu Gln Asp Ile Lys Ser Glu Asp
        275                 280                 285

Trp Ser Arg Phe Gly Ala Pro Phe Trp Pro Ala Val Ile Arg Ser Ala
    290                 295                 300

Phe Thr Trp Lys Gly Leu Thr Ser Leu Leu Ser Ser Gly Gln Lys Thr
305                 310                 315                 320

Ile Lys Gly Ala Leu Ala Met Pro Leu Met Ile Glu Gly Tyr Lys Lys
                325                 330                 335

Asp Leu Ile Lys Phe Ala Ile Ile Thr Cys Arg Lys Pro Glu
            340                 345                 350
```

<210> 30
<211> 310
<212> PRT
<213> Tagetes erecta

<400> 30

```
Ala Leu Ser Val Val Ala Ala Glu Val Pro Val Thr Val Thr Pro Ala
1               5                   10                  15

Thr Thr Lys Ala Glu Asp Val Glu Leu Lys Lys Gly Ile Ala Glu Phe
            20                  25                  30

Tyr Asp Glu Ser Ser Glu Met Trp Glu Asn Ile Trp Gly Glu His Met
        35                  40                  45

His His Gly Tyr Tyr Asn Thr Asn Ala Val Val Glu Leu Ser Asp His
    50                  55                  60
```

```
Arg Ser Ala Gln Ile Arg Met Ile Glu Gln Ala Leu Leu Phe Ala Ser
65                  70                  75                  80

Val Ser Asp Asp Pro Val Lys Lys Pro Arg Ser Ile Val Asp Val Gly
                85                  90                  95

Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu Ala Lys Lys Tyr Glu Ala
            100                 105                 110

Glu Cys His Gly Ile Thr Leu Ser Pro Val Gln Ala Glu Arg Ala Gln
        115                 120                 125

Ala Leu Ala Ala Ala Gln Gly Leu Ala Asp Lys Ala Ser Phe Gln Val
    130                 135                 140

Ala Asp Ala Leu Asp Gln Pro Phe Pro Asp Gly Lys Phe Asp Leu Val
145                 150                 155                 160

Trp Ser Met Glu Ser Gly Glu His Met Pro Asp Lys Leu Lys Phe Val
                165                 170                 175

Ser Glu Leu Val Arg Val Ala Ala Pro Gly Ala Thr Ile Ile Ile Val
            180                 185                 190

Thr Trp Cys His Arg Asp Leu Ser Pro Gly Glu Lys Ser Leu Arg Pro
        195                 200                 205

Asp Glu Glu Lys Ile Leu Lys Lys Ile Cys Ser Ser Phe Tyr Leu Pro
    210                 215                 220

Ala Trp Cys Ser Thr Ser Asp Tyr Val Lys Leu Leu Glu Ser Leu Ser
225                 230                 235                 240

Leu Gln Asp Ile Lys Ala Ala Asp Trp Ser Ala Asn Val Ala Pro Phe
                245                 250                 255

Trp Pro Ala Val Ile Lys Thr Ala Leu Ser Trp Lys Gly Ile Thr Ser
            260                 265                 270

Leu Leu Arg Ser Gly Trp Lys Ser Ile Arg Gly Ala Met Val Met Pro
        275                 280                 285

Leu Met Ile Glu Gly Phe Lys Lys Asp Ile Ile Lys Phe Ser Ile Ile
    290                 295                 300

Thr Cys Lys Lys Pro Glu
305                 310
```

<210> 31
<211> 354
<212> PRT
<213> Sorghum bicolor

<400> 31

```
Glu Arg Arg Ala Ala Gly Gly Arg Arg Glu Pro Leu Gly Gly Gly Ser
1               5                   10                  15

Val Pro Val Gly Ser His Tyr Tyr Tyr Arg Ala Pro Ser His Val Pro
            20                  25                  30

Arg Arg Ser Arg Pro Arg Gly Arg Gly Gly Val Val Ser Leu Arg Pro
        35                  40                  45

Met Ala Ser Ser Thr Ala Ala Gln Pro Pro Ala Pro Ala Pro Pro Gly
    50                  55                  60

Leu Lys Glu Gly Ile Ala Gly Leu Tyr Asp Glu Ser Ser Gly Leu Trp
65                  70                  75                  80

Glu Asn Ile Trp Gly Asp His Met His His Gly Phe Tyr Asp Ser Gly
                85                  90                  95

Glu Ala Ala Ser Met Ala Asp His Arg Arg Ala Gln Ile Arg Met Ile
            100                 105                 110

Glu Glu Ala Leu Ala Phe Ala Ala Val Pro Ser Pro Asp Asp Pro Glu
        115                 120                 125

Lys Ala Pro Lys Thr Ile Val Asp Val Gly Cys Gly Ile Gly Gly Ser
    130                 135                 140

Ser Arg Tyr Leu Ala Lys Lys Tyr Gly Ala Gln Cys Lys Gly Ile Thr
145                 150                 155                 160

Leu Ser Pro Val Gln Ala Glu Arg Gly Asn Ala Leu Ala Thr Ala Gln
                165                 170                 175

Gly Leu Ser Asp Gln Val Thr Leu Gln Val Ala Asp Ala Leu Glu Gln
            180                 185                 190

Pro Phe Pro Asp Gly Gln Phe Asp Leu Val Trp Ser Met Glu Ser Gly
        195                 200                 205

Glu His Met Pro Asp Lys Arg Lys Phe Val Ser Glu Leu Ala Arg Val
    210                 215                 220
```

```
Ala Ala Pro Gly Gly Thr Ile Ile Ile Val Thr Trp Cys His Arg Asn
225                 230                 235                 240

Leu Glu Pro Ser Glu Thr Ser Leu Lys Pro Asp Glu Leu Ser Leu Leu
                245                 250                 255

Lys Arg Ile Cys Asp Ala Tyr Tyr Leu Pro Asp Trp Cys Ser Pro Ser
            260                 265                 270

Asp Tyr Val Asn Ile Ala Lys Ser Leu Ser Leu Glu Asp Ile Lys Ala
        275                 280                 285

Ala Asp Trp Ser Glu Asn Val Ala Pro Phe Trp Pro Ala Val Ile Lys
    290                 295                 300

Ser Ala Leu Thr Trp Lys Gly Leu Thr Ser Leu Leu Thr Ser Gly Trp
305                 310                 315                 320

Lys Thr Ile Arg Gly Ala Met Val Met Pro Leu Met Ile Gln Gly Tyr
                325                 330                 335

Lys Lys Gly Leu Ile Lys Phe Thr Ile Ile Thr Cys Arg Lys Pro Gly
            340                 345                 350

Ala Ala
```

<210> 32
<211> 92
<212> PRT
<213> pea

<400> 32

```
Met Ala Ser Ser Met Leu Ser Ser Ala Thr Met Val Ala Ser Pro Ala
1               5                   10                  15

Gln Ala Thr Met Val Ala Pro Phe Asn Gly Leu Lys Ser Ser Ala Ala
            20                  25                  30

Phe Pro Ala Thr Arg Lys Ala Asn Asn Asp Ile Thr Ser Ile Thr Ser
        35                  40                  45

Asn Gly Gly Arg Val Asn Cys Met Gln Val Trp Pro Pro Ile Gly Lys
    50                  55                  60

Lys Lys Phe Glu Thr Leu Ser Tyr Leu Pro Asp Leu Thr Asp Ser Gly
65                  70                  75                  80

Gly Arg Val Asn Cys Met Gln Ala Asn Asn Asn Asn
                85                  90
```

<210> 33
<211> 301
<212> PRT
<213> Brassica napus

<400> 33
Met Val Ala Val Thr Ala Ala Ala Thr Ser Ser Val Glu Ala Leu Arg
1 5 10 15

Glu Gly Ile Ala Glu Phe Tyr Asn Glu Thr Ser Gly Leu Trp Glu Glu
20 25 30

Ile Trp Gly Asp His Met His His Gly Phe Tyr Asp Pro Asp Ser Ser
35 40 45

Val Gln Leu Ser Asp Ser Gly His Arg Glu Ala Gln Ile Arg Met Ile
50 55 60

Glu Glu Ser Leu Arg Phe Ala Gly Val Thr Glu Glu Glu Lys Lys Ile
65 70 75 80

Lys Arg Val Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr
85 90 95

Ile Ala Ser Lys Phe Gly Ala Glu Cys Ile Gly Ile Thr Leu Ser Pro
100 105 110

Val Gln Ala Lys Arg Ala Asn Asp Leu Ala Ala Ala Gln Ser Leu Ser
115 120 125

His Lys Val Ser Phe Gln Val Ala Asp Ala Leu Glu Gln Pro Phe Glu
130 135 140

Asp Gly Ile Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His Met
145 150 155 160

Pro Asp Lys Ala Lys Phe Val Lys Glu Leu Val Arg Val Ala Ala Pro
165 170 175

Gly Gly Arg Ile Ile Ile Val Thr Trp Cys His Arg Asn Leu Ser Pro
180 185 190

Gly Glu Glu Ala Leu Gln Pro Trp Glu Gln Asn Leu Leu Asp Arg Ile
195 200 205

Cys Lys Thr Phe Tyr Leu Pro Ala Trp Cys Ser Thr Ser Asp Tyr Val
210 215 220

```
Asp Leu Leu Gln Ser Leu Ser Leu Gln Asp Ile Lys Cys Ala Asp Trp
225                 230                 235                 240

Ser Glu Asn Val Ala Pro Phe Trp Pro Ala Val Ile Arg Thr Ala Leu
                245                 250                 255

Thr Trp Lys Gly Leu Val Ser Leu Leu Arg Ser Gly Met Lys Ser Ile
            260                 265                 270

Lys Gly Ala Leu Thr Met Pro Leu Met Ile Glu Gly Tyr Lys Lys Gly
        275                 280                 285

Val Ile Lys Phe Gly Ile Ile Thr Cys Gln Lys Pro Leu
    290                 295                 300
```

<210> 34
<211> 301
<212> PRT
<213> Brassica napus

```
<400> 34
Met Val Ala Val Thr Ala Ala Ala Thr Ser Ser Ala Glu Ala Leu Arg
1               5                   10                  15

Glu Gly Ile Ala Glu Phe Tyr Asn Glu Thr Ser Gly Leu Trp Glu Glu
            20                  25                  30

Ile Trp Gly Asp His Met His His Gly Phe Tyr Asp Pro Asp Ser Ser
        35                  40                  45

Val Gln Leu Ser Asp Ser Gly His Arg Glu Ala Gln Ile Arg Met Ile
    50                  55                  60

Glu Glu Ser Leu Arg Phe Ala Gly Val Thr Glu Glu Glu Lys Lys Ile
65                  70                  75                  80

Lys Arg Val Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr
                85                  90                  95

Ile Ala Ser Lys Phe Gly Ala Glu Cys Ile Gly Ile Thr Leu Ser Pro
            100                 105                 110

Val Gln Ala Lys Arg Ala Asn Asp Leu Ala Thr Ala Gln Ser Leu Ser
        115                 120                 125

His Lys Val Ser Phe Gln Val Ala Asp Ala Leu Asp Gln Pro Phe Glu
    130                 135                 140

Asp Gly Ile Ser Asp Leu Val Trp Ser Met Glu Ser Gly Glu His Met
145                 150                 155                 160
```

```
Pro Asp Lys Ala Lys Phe Val Lys Glu Leu Val Arg Val Thr Ala Pro
                165                 170                 175

Gly Gly Arg Ile Ile Ile Val Thr Trp Cys His Arg Asn Leu Ser Gln
            180                 185                 190

Gly Glu Glu Ser Leu Gln Pro Trp Glu Gln Asn Leu Leu Asp Arg Ile
        195                 200                 205

Cys Lys Thr Phe Tyr Leu Pro Ala Trp Cys Ser Thr Thr Asp Tyr Val
    210                 215                 220

Glu Leu Leu Gln Ser Leu Ser Leu Gln Asp Ile Lys Tyr Ala Asp Trp
225                 230                 235                 240

Ser Glu Asn Val Ala Pro Phe Trp Pro Ala Val Ile Arg Thr Ala Leu
                245                 250                 255

Thr Trp Lys Gly Leu Val Ser Leu Leu Arg Ser Gly Met Lys Ser Ile
            260                 265                 270

Lys Gly Ala Leu Thr Met Pro Leu Met Ile Glu Gly Tyr Lys Lys Gly
        275                 280                 285

Val Ile Lys Phe Gly Ile Ile Thr Cys Gln Lys Pro Leu
    290                 295                 300
```

<210> 35
<211> 315
<212> PRT
<213> Cuphea pulcherrima

<400> 35

```
Met Ala Thr Ala Asp Lys Thr Gln Ser Thr Asp Thr Ser Asn Glu Gly
1               5                   10                  15

Val Val Ser Tyr Asp Thr Gln Val Leu Gln Lys Gly Ile Ala Glu Phe
            20                  25                  30

Tyr Asp Glu Ser Ser Gly Ile Trp Glu Asp Ile Trp Gly Asp His Met
        35                  40                  45

His His Gly Tyr Tyr Asp Gly Ser Thr Pro Val Ser Leu Pro Asp His
    50                  55                  60

Arg Ser Ala Gln Ile Arg Met Ile Asp Glu Ala Leu Arg Phe Ala Ser
65                  70                  75                  80
```

```
Val Pro Ser Gly Glu Glu Asp Glu Ser Lys Ser Lys Ile Pro Lys Arg
                85                  90                  95

Ile Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu Ala
            100                 105                 110

Arg Lys Tyr Gly Ala Glu Cys Arg Gly Ile Thr Leu Ser Pro Val Gln
        115                 120                 125

Ala Glu Arg Gly Asn Ser Leu Ala Arg Ser Gln Gly Leu Ser Asp Lys
    130                 135                 140

Val Ser Phe Gln Val Ala Asp Ala Leu Ala Gln Pro Phe Pro Asp Gly
145                 150                 155                 160

Gln Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro Asp
                165                 170                 175

Lys Ser Lys Phe Val Asn Glu Leu Val Arg Val Ala Ala Pro Gly Gly
            180                 185                 190

Thr Ile Ile Ile Val Thr Trp Cys His Arg Asp Leu Arg Glu Asp Glu
        195                 200                 205

Asp Ala Leu Gln Pro Arg Glu Lys Glu Ile Leu Asp Lys Ile Cys Asn
    210                 215                 220

Pro Phe Tyr Leu Pro Ala Trp Cys Ser Ala Ala Asp Tyr Val Lys Leu
225                 230                 235                 240

Leu Gln Ser Leu Asp Val Glu Asp Ile Lys Ser Ala Asp Trp Thr Pro
                245                 250                 255

Tyr Val Ala Pro Phe Trp Pro Ala Val Leu Lys Ser Ala Phe Thr Ile
            260                 265                 270

Lys Gly Phe Val Ser Leu Leu Arg Ser Gly Met Lys Thr Ile Lys Gly
        275                 280                 285

Ala Phe Ala Met Pro Leu Met Ile Glu Gly Tyr Lys Lys Gly Val Ile
    290                 295                 300

Lys Phe Ser Ile Ile Thr Cys Arg Lys Pro Glu
305                 310                 315
```

<210> 36
<211> 299
<212> PRT
<213> Gossypium hirsutum

<400> 36

```
Met Val Lys Ala Ala Ala Ser Ser Leu Ser Thr Thr Thr Leu Gln Glu
1               5                   10                  15

Gly Ile Ala Glu Phe Tyr Asp Glu Ser Ser Gly Ile Trp Glu Asp Ile
            20                  25                  30

Trp Gly Asp His Met His His Gly Tyr Tyr Glu Pro Gly Ser Asp Ile
        35                  40                  45

Ser Gly Ser Asp His Arg Ala Ala Gln Ile Arg Met Val Glu Glu Ser
    50                  55                  60

Leu Arg Phe Ala Gly Ile Ser Glu Asp Pro Ala Asn Arg Pro Lys Arg
65                  70                  75                  80

Ile Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu Ala
                85                  90                  95

Arg Lys Tyr Gly Ala Lys Cys Gln Gly Ile Thr Leu Ser Pro Val Gln
            100                 105                 110

Ala Gly Arg Ala Asn Ala Leu Ala Asn Ala Gln Gly Leu Ala Glu Gln
        115                 120                 125

Val Cys Phe Glu Val Ala Asp Ala Leu Asn Gln Pro Phe Pro Asp Asp
    130                 135                 140

Gln Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro Asp
145                 150                 155                 160

Lys Pro Lys Phe Val Lys Glu Leu Val Val Ala Ala Pro Gly Gly Thr
                165                 170                 175

Ile Ile Val Val Thr Trp Cys His Arg Asp Leu Gly Pro Ser Glu Glu
            180                 185                 190

Ser Leu Gln Pro Trp Glu Gln Lys Leu Leu Asn Arg Ile Cys Asp Ala
        195                 200                 205

Tyr Tyr Leu Pro Glu Trp Cys Ser Thr Ser Asp Tyr Val Lys Leu Phe
    210                 215                 220

Gln Ser Leu Ser Leu Gln Asp Ile Lys Ala Gly Asp Trp Thr Glu Asn
225                 230                 235                 240
```

Val Ala Pro Phe Trp Pro Ala Val Ile Arg Ser Ala Leu Thr Trp Lys
245 250 255

Gly Phe Thr Ser Leu Leu Arg Ser Gly Leu Lys Thr Ile Lys Gly Ala
260 265 270

Leu Val Met Pro Leu Met Ile Glu Gly Phe Gln Lys Gly Val Ile Lys
275 280 285

Phe Ala Ile Ile Ala Cys Arg Lys Pro Ala Glu
290 295

<210> 37
<211> 311
<212> PRT
<213> Tagetes erecta

<400> 37
Met Ala Leu Ser Val Val Ala Ala Glu Val Pro Val Thr Val Thr Pro
1 5 10 15

Ala Thr Thr Lys Ala Glu Asp Val Glu Leu Lys Lys Gly Ile Ala Glu
20 25 30

Phe Tyr Asp Glu Ser Ser Glu Met Trp Glu Asn Ile Trp Gly Glu His
35 40 45

Met His His Gly Tyr Tyr Asn Thr Asn Ala Val Val Glu Leu Ser Asp
50 55 60

His Arg Ser Ala Gln Ile Arg Met Ile Glu Gln Ala Leu Leu Phe Ala
65 70 75 80

Ser Val Ser Asp Asp Pro Val Lys Lys Pro Arg Ser Ile Val Asp Val
85 90 95

Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu Ala Lys Lys Tyr Glu
100 105 110

Ala Glu Cys His Gly Ile Thr Leu Ser Pro Val Gln Ala Glu Arg Ala
115 120 125

Gln Ala Leu Ala Ala Ala Gln Gly Leu Ala Asp Lys Ala Ser Phe Gln
130 135 140

Val Ala Asp Ala Leu Asp Gln Pro Phe Pro Asp Gly Lys Phe Asp Leu
145 150 155 160

```
Val Trp Ser Met Glu Ser Gly Glu His Met Pro Asp Lys Leu Lys Phe
                165                 170                 175

Val Ser Glu Leu Val Arg Val Ala Ala Pro Gly Ala Thr Ile Ile Ile
            180                 185                 190

Val Thr Trp Cys His Arg Asp Leu Ser Pro Gly Glu Lys Ser Leu Arg
        195                 200                 205

Pro Asp Glu Glu Lys Ile Leu Lys Lys Ile Cys Ser Ser Phe Tyr Leu
    210                 215                 220

Pro Ala Trp Cys Ser Thr Ser Asp Tyr Val Lys Leu Leu Glu Ser Leu
225                 230                 235                 240

Ser Leu Gln Asp Ile Lys Ala Ala Asp Trp Ser Ala Asn Val Ala Pro
                245                 250                 255

Phe Trp Pro Ala Val Ile Lys Thr Ala Leu Ser Trp Lys Gly Ile Thr
            260                 265                 270

Ser Leu Leu Arg Ser Gly Trp Lys Ser Ile Arg Gly Ala Met Val Met
        275                 280                 285

Pro Leu Met Ile Glu Gly Phe Lys Lys Asp Ile Ile Lys Phe Ser Ile
    290                 295                 300

Ile Thr Cys Lys Lys Pro Glu
305                 310
```

<210> 38
<211> 305
<212> PRT
<213> Zea mays

<400> 38

```
Met Ala Ser Ser Thr Ala Gln Ala Pro Ala Thr Ala Pro Pro Gly Leu
1               5                   10                  15

Lys Glu Gly Ile Ala Gly Leu Tyr Asp Glu Ser Ser Gly Leu Trp Glu
            20                  25                  30

Asn Ile Trp Gly Asp His Met His His Gly Phe Tyr Asp Ser Ser Glu
        35                  40                  45

Ala Ala Ser Met Ala Asp His Arg Arg Ala Gln Ile Arg Met Ile Glu
    50                  55                  60

Glu Ala Leu Ala Phe Ala Gly Val Pro Ala Ser Asp Asp Pro Glu Lys
65                  70                  75                  80
```

Thr Pro Lys Thr Ile Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser
85 90 95

Arg Tyr Leu Ala Lys Lys Tyr Gly Ala Gln Cys Thr Gly Ile Thr Leu
100 105 110

Ser Pro Val Gln Ala Glu Arg Gly Asn Ala Leu Ala Ala Ala Gln Gly
115 120 125

Leu Ser Asp Gln Val Thr Leu Gln Val Ala Asp Ala Leu Glu Gln Pro
130 135 140

Phe Pro Asp Gly Gln Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu
145 150 155 160

His Met Pro Asp Lys Arg Lys Phe Val Ser Glu Leu Ala Arg Val Ala
165 170 175

Ala Pro Gly Gly Thr Ile Ile Ile Val Thr Trp Cys His Arg Asn Leu
180 185 190

Asp Pro Ser Glu Thr Ser Leu Lys Pro Asp Glu Leu Ser Leu Leu Arg
195 200 205

Arg Ile Cys Asp Ala Tyr Tyr Leu Pro Asp Trp Cys Ser Pro Ser Asp
210 215 220

Tyr Val Asn Ile Ala Lys Ser Leu Ser Leu Glu Asp Ile Lys Thr Ala
225 230 235 240

Asp Trp Ser Glu Asn Val Ala Pro Phe Trp Pro Ala Val Ile Lys Ser
245 250 255

Ala Leu Thr Trp Lys Gly Phe Thr Ser Leu Leu Thr Thr Gly Trp Lys
260 265 270

Thr Ile Arg Gly Ala Met Val Met Pro Leu Met Ile Gln Gly Tyr Lys
275 280 285

Lys Gly Leu Ile Lys Phe Thr Ile Ile Thr Cys Arg Lys Pro Gly Ala
290 295 300

Ala
305

<210> 39
<211> 280
<212> PRT
<213> Nostoc punctiforme

<400> 39
Met Ser Ala Thr Leu Tyr Gln Gln Ile Gln Gln Phe Tyr Asp Ala Ser
1 5 10 15

Ser Gly Leu Trp Glu Gln Ile Trp Gly Glu His Met His His Gly Tyr
20 25 30

Tyr Gly Ala Asp Gly Thr Gln Lys Lys Asp Arg Arg Gln Ala Gln Ile
35 40 45

Asp Leu Ile Glu Glu Leu Leu Asn Trp Ala Gly Val Gln Ala Ala Glu
50 55 60

Asp Ile Leu Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Leu Tyr Leu
65 70 75 80

Ala Gln Lys Phe Asn Ala Lys Ala Thr Gly Ile Thr Leu Ser Pro Val
85 90 95

Gln Ala Ala Arg Ala Thr Glu Arg Ala Leu Glu Ala Asn Leu Ser Leu
100 105 110

Arg Thr Gln Phe Gln Val Ala Asn Ala Gln Ala Met Pro Phe Ala Asp
115 120 125

Asp Ser Phe Asp Leu Val Trp Ser Leu Glu Ser Gly Glu His Met Pro
130 135 140

Asp Lys Thr Lys Phe Leu Gln Glu Cys Tyr Arg Val Leu Lys Pro Gly
145 150 155 160

Gly Lys Leu Ile Met Val Thr Trp Cys His Arg Pro Thr Asp Glu Ser
165 170 175

Pro Leu Thr Ala Asp Glu Glu Lys His Leu Gln Asp Ile Tyr Arg Val
180 185 190

Tyr Cys Leu Pro Tyr Val Ile Ser Leu Pro Glu Tyr Glu Ala Ile Ala
195 200 205

His Gln Leu Pro Leu His Asn Ile Arg Thr Ala Asp Trp Ser Thr Ala
210 215 220

Val Ala Pro Phe Trp Asn Val Val Ile Asp Ser Ala Phe Thr Pro Gln
225 230 235 240

```
Ala Leu Trp Gly Leu Leu Asn Ala Gly Trp Thr Thr Ile Gln Gly Ala
                245                 250                 255

Leu Ser Leu Gly Leu Met Arg Arg Gly Tyr Glu Arg Gly Leu Ile Arg
            260                 265                 270

Phe Gly Leu Leu Cys Gly Asn Lys
        275                 280
```

<210> 40
<211> 280
<212> PRT
<213> Anabaena sp.

<400> 40

```
Met Ser Ala Thr Leu Tyr Gln Gln Ile Gln Gln Phe Tyr Asp Ala Ser
1               5                   10                  15

Ser Gly Leu Trp Glu Glu Ile Trp Gly Glu His Met His His Gly Tyr
            20                  25                  30

Tyr Gly Ala Asp Gly Thr Glu Gln Lys Asn Arg Arg Gln Ala Gln Ile
        35                  40                  45

Asp Leu Ile Glu Glu Leu Leu Thr Trp Ala Gly Val Gln Thr Ala Glu
    50                  55                  60

Asn Ile Leu Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Leu Tyr Leu
65                  70                  75                  80

Ala Gly Lys Leu Asn Ala Lys Ala Thr Gly Ile Thr Leu Ser Pro Val
                85                  90                  95

Gln Ala Ala Arg Ala Thr Glu Arg Ala Lys Glu Ala Gly Leu Ser Gly
            100                 105                 110

Arg Ser Gln Phe Leu Val Ala Asn Ala Gln Ala Met Pro Phe Asp Asp
        115                 120                 125

Asn Ser Phe Asp Leu Val Trp Ser Leu Glu Ser Gly Glu His Met Pro
    130                 135                 140

Asp Lys Thr Lys Phe Leu Gln Glu Cys Tyr Arg Val Leu Lys Pro Gly
145                 150                 155                 160

Gly Lys Leu Ile Met Val Thr Trp Cys His Arg Pro Thr Asp Lys Thr
                165                 170                 175
```

```
Pro Leu Thr Ala Asp Glu Lys Lys His Leu Glu Asp Ile Tyr Arg Val
            180                 185                 190

Tyr Cys Leu Pro Tyr Val Ile Ser Leu Pro Glu Tyr Glu Ala Ile Ala
        195                 200                 205

Arg Gln Leu Pro Leu Asn Asn Ile Arg Thr Ala Asp Trp Ser Gln Ser
    210                 215                 220

Val Ala Gln Phe Trp Asn Ile Val Ile Asp Ser Ala Phe Thr Pro Gln
225                 230                 235                 240

Ala Ile Phe Gly Leu Leu Arg Ala Gly Trp Thr Thr Ile Gln Gly Ala
                245                 250                 255

Leu Ser Leu Gly Leu Met Arg Arg Gly Tyr Glu Arg Gly Leu Ile Arg
            260                 265                 270

Phe Gly Leu Leu Cys Gly Asp Lys
        275                 280
```

<210> 41
<211> 317
<212> PRT
<213> Synechocystis PCC 6803

<400> 41

```
Met Val Tyr His Val Arg Pro Lys His Ala Leu Phe Leu Ala Phe Tyr
1               5                   10                  15

Cys Tyr Phe Ser Leu Leu Thr Met Ala Ser Ala Thr Ile Ala Ser Ala
            20                  25                  30

Asp Leu Tyr Glu Lys Ile Lys Asn Phe Tyr Asp Asp Ser Ser Gly Leu
        35                  40                  45

Trp Glu Asp Val Trp Gly Glu His Met His His Gly Tyr Tyr Gly Pro
    50                  55                  60

His Gly Thr Tyr Arg Ile Asp Arg Arg Gln Ala Gln Ile Asp Leu Ile
65                  70                  75                  80

Lys Glu Leu Leu Ala Trp Ala Val Pro Gln Asn Ser Ala Lys Pro Arg
                85                  90                  95

Lys Ile Leu Asp Leu Gly Cys Gly Ile Gly Gly Ser Ser Leu Tyr Leu
            100                 105                 110

Ala Gln Gln His Gln Ala Glu Val Met Gly Ala Ser Leu Ser Pro Val
        115                 120                 125
```

```
Gln Val Glu Arg Ala Gly Glu Arg Ala Arg Ala Leu Gly Leu Gly Ser
    130                 135                 140

Thr Cys Gln Phe Gln Val Ala Asn Ala Leu Asp Leu Pro Phe Ala Ser
145                 150                 155                 160

Asp Ser Phe Asp Trp Val Trp Ser Leu Glu Ser Gly Glu His Met Pro
                165                 170                 175

Asn Lys Ala Gln Phe Leu Gln Glu Ala Trp Arg Val Leu Lys Pro Gly
            180                 185                 190

Gly Arg Leu Ile Leu Ala Thr Trp Cys His Arg Pro Ile Asp Pro Gly
        195                 200                 205

Asn Gly Pro Leu Thr Ala Asp Glu Arg Arg His Leu Gln Ala Ile Tyr
    210                 215                 220

Asp Val Tyr Cys Leu Pro Tyr Val Val Ser Leu Pro Asp Tyr Glu Ala
225                 230                 235                 240

Ile Ala Arg Glu Cys Gly Phe Gly Glu Ile Lys Thr Ala Asp Trp Ser
                245                 250                 255

Val Ala Val Ala Pro Phe Trp Asp Arg Val Ile Glu Ser Ala Phe Asp
            260                 265                 270

Pro Arg Val Leu Trp Ala Leu Gly Gln Ala Gly Pro Lys Ile Ile Asn
        275                 280                 285

Ala Ala Leu Cys Leu Arg Leu Met Lys Trp Gly Tyr Glu Arg Gly Leu
    290                 295                 300

Val Arg Phe Gly Leu Leu Thr Gly Ile Lys Pro Leu Val
305                 310                 315
```

<210> 42
<211> 957
<212> DNA
<213> Synechocystis PCC 6803

<400> 42
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gaccaatgga cagaggacgg cattttggaa tattactggg gcgaccatat ccacctcggc 180

cattatggcg atccgccagt ggccaaggat ttcatccaat cgaaaattga ttttgtccat 240

gccatggccc agtggggcgg attagataca cttccccccg gcacaacggt attggatgtg 300

ggttgcggca ttggcggtag cagtcgcatt ctcgccaaag attatggttt taacgttacc 360

ggcatcacca ttagtcccca acaggtgaaa cgggcgacgg aattaactcc tcccgatgtg 420

acggccaagt ttgcggtgga cgatgctatg gctttgtctt ttcctgacgg tagtttcgac 480

gtagtttggt cggtggaagc agggccccac atgcctgaca aagctgtgtt tgccaaggaa 540

ttactgcggg tcgtgaaacc agggggcatt ctggtggtgg cggattggaa tcaacgggac 600

gatcgccaag tgcccctcaa cttctgggaa aaaccagtga tgcgacaact gttggatcaa 660

tggtcccacc ctgcctttgc cagcattgaa ggttttgcgg aaaatttgga agccacgggt 720

ttggtggagg gccaggtgac tactgctgat tggactgtac cgaccctccc cgcttggttg 780

gataccattt ggcagggcat tatccggccc cagggctggt tacaatacgg cattcgtggg 840

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ggactttgtc gcttcggtat gttcaaagca gtgcgaaaaa acgccactca agcttaa 957

<210> 43
<211> 993
<212> DNA
<213> Anabaena sp.

<400> 43
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ttatatctca ttactgctag acgttatcaa tcatctaact ccgtagccaa ttcctacgac 120

cagtggactg aagacggtat tttagagttt tactggggcg aacatatcca tttaggtcat 180

tatggttcgc cacctcaaag aaaggatttt ctggtggcta aatctgattt tgtccatgaa 240

atggtgcgtt ggggtggttt ggataaacta ccccctggta ctaccttgtt agatgttggt 300

tgtggaattg ggggtagtag tcgcattttg gcacgggatt atggatttgc cgttacaggt 360

atcaccatca gcccccaaca agtccaacgc gctcaagagt taacaccaca ggaactgaat 420

gcacagtttt tggtggatga tgcaatggcg ctttccttcc cagataatag ttttgatgta 480

gtttggtcaa ttgaagctgg cccacatatg ccagataaag ccatttttgc caaagaattg 540

atgcgggtac taaagcctgg tggaatcatg gttttagccg actggaatca gcgagacgat 600

cgccaaaaac ccctcaattt ttgggagaaa ccagtaatgc agcaactact agatcagtgg 660

tctcatccag ctttttccag catcgaaggc ttttctgagc ttttggcagc gacgggatta 720

gtagaagggg aggtaatcac cgcagactgg acgaaacaaa cactcccctc ttggcttgat 780

tctatctggc aaggaatagt tagaccagaa ggattagtgc gttttggtct atctggtttc 840

attaaatctc tgcgagaagt gcctacccta ctactgatga ggctggcatt cggtacagga 900

ctctgtagat ttgggatgtt ccgcgcttta cgagctgaca ctgtaagatc atcagcagaa 960

cagacatctg cgatcaaggt tgctcaaaag taa 993

<210> 44
<211> 930
<212> DNA
<213> Synechococcus sp. MT1

<400> 44
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accgatgacc aactgctgga acggctctgg ggagaccatg tccacctggg gcattacgga 180

aacccgccag gttctgtcga cttccgccag gccaaggagg cttttgtgca cgagctggtg 240

cgctggagcg ggctcgacca actacctcga ggcagtcggg tgttggatgt gggttgcggc 300

atcggcggca gtgcccggat cctggccagg gattacggct tggacgtgct cggggtgagc 360

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acggtggagg cggggcccca catgccagac aagcagcgtt tcgctgatga gttgctgcgg 540

gtactccggc ccgggggctg cttagccgcc gctgattgga accgccgcgc ccccaaggat 600

ggcgccatga acagcaccga acgctgggtg atgcggcagt tgttgaatca atgggcgcat 660

ccggaattcg ccagcatctc cggcttccgg gccaacctcg aagccagccc tcaccagcgg 720

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ggcctgcgag agaccccgac gctgctgttg atgcattggg cctttgccac agggttgatg 900

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<210> 45
<211> 936
<212> DNA
<213> Prochlorococcus marinus

<400> 45
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atactttgga gaattaatac tagaaaatat atttcttcga gaactgtagc tacagcatat 120

gattcctgga ctcaagataa attactagaa agattatggg gagaacatat acatctaggt 180

ttctatcctc taaataaaaa tattgatttt agagaggcta aagttcaatt tgtacatgag 240

ttagtaagtt ggagtggttt agataaatta ccaagaggtt ctaggatttt agatgtcggt 300

tgcggaatag gtggaagttc tagaattctc gccaattatt atggatttaa tgtcactgga 360

ataactatta gtccagctca agtaaaaaga gcaaaagaac ttactcctta tgaatgtaaa 420

tgcaacttca aagttatgga tgctttggat ttgaaatttg aagagggaat atttgatggt 480

gtttggagtg ttgaggcagg agcccatatg aataataaaa ctaaatttgc agatcaaatg 540

ttaagaactt taagacctgg aggatattta gcattggctg attggaattc aagagattta 600

caaaagcaac ccccatccat gattgaaaaa ataatcttaa aacaattact tgaacagtgg 660

gtacatccta aatttattag tatcaatgaa ttcagtagta ttcttataaa taacaaaaat 720

agttcaggtc aagttatatc ctctaattgg aattctttta caaatccctc ttggtttgat 780

tcaatatttg aaggaatgag aagacctaat tcaattttat cccttggtcc aggagcaatt 840

ataaagtcta tcagagagat acctacaata cttttaatgg attgggcctt taaaaaaggt 900

ttaatggaat ttggagttta taaatgtaga ggttaa 936

<210> 46
<211> 318
<212> PRT
<213> Synechocystis PCC6803

<400> 46

```
Met Pro Glu Tyr Leu Leu Leu Pro Ala Gly Leu Ile Ser Leu Ser Leu
1               5                  10                  15

Ala Ile Ala Ala Gly Leu Tyr Leu Leu Thr Ala Arg Gly Tyr Gln Ser
            20                  25                  30

Ser Asp Ser Val Ala Asn Ala Tyr Asp Gln Trp Thr Glu Asp Gly Ile
        35                  40                  45

Leu Glu Tyr Tyr Trp Gly Asp His Ile His Leu Gly His Tyr Gly Asp
    50                  55                  60

Pro Pro Val Ala Lys Asp Phe Ile Gln Ser Lys Ile Asp Phe Val His
65                  70                  75                  80

Ala Met Ala Gln Trp Gly Gly Leu Asp Thr Leu Pro Pro Gly Thr Thr
                85                  90                  95

Val Leu Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Ile Leu Ala
            100                 105                 110

Lys Asp Tyr Gly Phe Asn Val Thr Gly Ile Thr Ile Ser Pro Gln Gln
        115                 120                 125

Val Lys Arg Ala Thr Glu Leu Thr Pro Pro Asp Val Thr Ala Lys Phe
    130                 135                 140

Ala Val Asp Asp Ala Met Ala Leu Ser Phe Pro Asp Gly Ser Phe Asp
145                 150                 155                 160

Val Val Trp Ser Val Glu Ala Gly Pro His Met Pro Asp Lys Ala Val
                165                 170                 175
```

```
Phe Ala Lys Glu Leu Leu Arg Val Val Lys Pro Gly Gly Ile Leu Val
            180                 185                 190

Val Ala Asp Trp Asn Gln Arg Asp Asp Arg Gln Val Pro Leu Asn Phe
        195                 200                 205

Trp Glu Lys Pro Val Met Arg Gln Leu Leu Asp Gln Trp Ser His Pro
    210                 215                 220

Ala Phe Ala Ser Ile Glu Gly Phe Ala Glu Asn Leu Glu Ala Thr Gly
225                 230                 235                 240

Leu Val Glu Gly Gln Val Thr Thr Ala Asp Trp Thr Val Pro Thr Leu
                245                 250                 255

Pro Ala Trp Leu Asp Thr Ile Trp Gln Gly Ile Ile Arg Pro Gln Gly
            260                 265                 270

Trp Leu Gln Tyr Gly Ile Arg Gly Phe Ile Lys Ser Val Arg Glu Val
        275                 280                 285

Pro Thr Ile Leu Leu Met Arg Leu Ala Phe Gly Val Gly Leu Cys Arg
    290                 295                 300

Phe Gly Met Phe Lys Ala Val Arg Lys Asn Ala Thr Gln Ala
305                 310                 315
```

<210> 47
<211> 330
<212> PRT
<213> Anabaena sp.

<400> 47

```
Met Ser Trp Leu Phe Ser Thr Leu Val Phe Phe Leu Thr Leu Leu Thr
1               5                   10                  15

Ala Gly Ile Ala Leu Tyr Leu Ile Thr Ala Arg Arg Tyr Gln Ser Ser
            20                  25                  30

Asn Ser Val Ala Asn Ser Tyr Asp Gln Trp Thr Glu Asp Gly Ile Leu
        35                  40                  45

Glu Phe Tyr Trp Gly Glu His Ile His Leu Gly His Tyr Gly Ser Pro
    50                  55                  60

Pro Gln Arg Lys Asp Phe Leu Val Ala Lys Ser Asp Phe Val His Glu
65                  70                  75                  80
```

```
Met Val Arg Trp Gly Gly Leu Asp Lys Leu Pro Pro Gly Thr Thr Leu
                85                  90                  95

Leu Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Ile Leu Ala Arg
            100                 105                 110

Asp Tyr Gly Phe Ala Val Thr Gly Ile Thr Ile Ser Pro Gln Gln Val
        115                 120                 125

Gln Arg Ala Gln Glu Leu Thr Pro Gln Glu Leu Asn Ala Gln Phe Leu
    130                 135                 140

Val Asp Asp Ala Met Ala Leu Ser Phe Pro Asp Asn Ser Phe Asp Val
145                 150                 155                 160

Val Trp Ser Ile Glu Ala Gly Pro His Met Pro Asp Lys Ala Ile Phe
                165                 170                 175

Ala Lys Glu Leu Met Arg Val Leu Lys Pro Gly Gly Ile Met Val Leu
            180                 185                 190

Ala Asp Trp Asn Gln Arg Asp Asp Arg Gln Lys Pro Leu Asn Phe Trp
        195                 200                 205

Glu Lys Pro Val Met Gln Gln Leu Leu Asp Gln Trp Ser His Pro Ala
    210                 215                 220

Phe Ser Ser Ile Glu Gly Phe Ser Glu Leu Leu Ala Ala Thr Gly Leu
225                 230                 235                 240

Val Glu Gly Glu Val Ile Thr Ala Asp Trp Thr Lys Gln Thr Leu Pro
                245                 250                 255

Ser Trp Leu Asp Ser Ile Trp Gln Gly Ile Val Arg Pro Glu Gly Leu
            260                 265                 270

Val Arg Phe Gly Leu Ser Gly Phe Ile Lys Ser Leu Arg Glu Val Pro
        275                 280                 285

Thr Leu Leu Leu Met Arg Leu Ala Phe Gly Thr Gly Leu Cys Arg Phe
    290                 295                 300

Gly Met Phe Arg Ala Leu Arg Ala Asp Thr Val Arg Ser Ser Ala Glu
305                 310                 315                 320

Gln Thr Ser Ala Ile Lys Val Ala Gln Lys
                325                 330
```

<210> 48
<211> 309
<212> PRT
<213> Synechococcus sp.

<400> 48

```
Met Leu Ala Gly Leu Leu Leu Leu Thr Gly Ala Ala Gly Ala Thr Ala
1               5                  10                  15

Leu Leu Ile Trp Leu Gln Arg Asp Arg Arg Tyr His Ser Ser Asp Ser
            20                  25                  30

Val Ala Ala Ala Tyr Asp Ala Trp Thr Asp Asp Gln Leu Leu Glu Arg
        35                  40                  45

Leu Trp Gly Asp His Val His Leu Gly His Tyr Gly Asn Pro Pro Gly
    50                  55                  60

Ser Val Asp Phe Arg Gln Ala Lys Glu Ala Phe Val His Glu Leu Val
65                  70                  75                  80

Arg Trp Ser Gly Leu Asp Gln Leu Pro Arg Gly Ser Arg Val Leu Asp
                85                  90                  95

Val Gly Cys Gly Ile Gly Gly Ser Ala Arg Ile Leu Ala Arg Asp Tyr
            100                 105                 110

Gly Leu Asp Val Leu Gly Val Ser Ile Ser Pro Ala Gln Ile Arg Arg
        115                 120                 125

Ala Thr Glu Leu Thr Pro Ala Gly Leu Ser Cys Arg Phe Glu Val Met
    130                 135                 140

Asp Ala Leu Asn Leu Gln Leu Pro Asp Arg Gln Phe Asp Ala Val Trp
145                 150                 155                 160

Thr Val Glu Ala Gly Pro His Met Pro Asp Lys Gln Arg Phe Ala Asp
                165                 170                 175

Glu Leu Leu Arg Val Leu Arg Pro Gly Gly Cys Leu Ala Ala Ala Asp
            180                 185                 190

Trp Asn Arg Arg Ala Pro Lys Asp Gly Ala Met Asn Ser Thr Glu Arg
        195                 200                 205

Trp Val Met Arg Gln Leu Leu Asn Gln Trp Ala His Pro Glu Phe Ala
    210                 215                 220

Ser Ile Ser Gly Phe Arg Ala Asn Leu Glu Ala Ser Pro His Gln Arg
225                 230                 235                 240
```

```
Gly Leu Ile Ser Thr Gly Asp Trp Thr Leu Ala Thr Leu Pro Ser Trp
                245                 250                 255

Phe Asp Ser Ile Ala Glu Gly Leu Arg Arg Pro Trp Ala Val Leu Gly
            260                 265                 270

Leu Gly Pro Lys Ala Val Leu Gln Gly Leu Arg Glu Thr Pro Thr Leu
        275                 280                 285

Leu Leu Met His Trp Ala Phe Ala Thr Gly Leu Met Gln Phe Gly Val
    290                 295                 300

Phe Arg Leu Ser Arg
305
```

<210> 49
<211> 311
<212> PRT
<213> Prochlorococcus marinus

<400> 49

```
Met Ser Ile Phe Leu Ile Ser Ser Leu Val Ile Phe Leu Thr Leu Leu
1               5                   10                  15

Phe Ser Ser Leu Ile Leu Trp Arg Ile Asn Thr Arg Lys Tyr Ile Ser
            20                  25                  30

Ser Arg Thr Val Ala Thr Ala Tyr Asp Ser Trp Thr Gln Asp Lys Leu
        35                  40                  45

Leu Glu Arg Leu Trp Gly Glu His Ile His Leu Gly Phe Tyr Pro Leu
    50                  55                  60

Asn Lys Asn Ile Asp Phe Arg Glu Ala Lys Val Gln Phe Val His Glu
65                  70                  75                  80

Leu Val Ser Trp Ser Gly Leu Asp Lys Leu Pro Arg Gly Ser Arg Ile
                85                  90                  95

Leu Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Ile Leu Ala Asn
            100                 105                 110

Tyr Tyr Gly Phe Asn Val Thr Gly Ile Thr Ile Ser Pro Ala Gln Val
        115                 120                 125

Lys Arg Ala Lys Glu Leu Thr Pro Tyr Glu Cys Lys Cys Asn Phe Lys
    130                 135                 140
```

```
Val Met Asp Ala Leu Asp Leu Lys Phe Glu Glu Gly Ile Phe Asp Gly
145                 150                 155                 160

Val Trp Ser Val Glu Ala Gly Ala His Met Asn Asn Lys Thr Lys Phe
                165                 170                 175

Ala Asp Gln Met Leu Arg Thr Leu Arg Pro Gly Gly Tyr Leu Ala Leu
            180                 185                 190

Ala Asp Trp Asn Ser Arg Asp Leu Gln Lys Gln Pro Pro Ser Met Ile
        195                 200                 205

Glu Lys Ile Ile Leu Lys Gln Leu Leu Glu Gln Trp Val His Pro Lys
    210                 215                 220

Phe Ile Ser Ile Asn Glu Phe Ser Ser Ile Leu Ile Asn Asn Lys Asn
225                 230                 235                 240

Ser Ser Gly Gln Val Ile Ser Ser Asn Trp Asn Ser Phe Thr Asn Pro
                245                 250                 255

Ser Trp Phe Asp Ser Ile Phe Glu Gly Met Arg Arg Pro Asn Ser Ile
            260                 265                 270

Leu Ser Leu Gly Pro Gly Ala Ile Ile Lys Ser Ile Arg Glu Ile Pro
        275                 280                 285

Thr Ile Leu Leu Met Asp Trp Ala Phe Lys Lys Gly Leu Met Glu Phe
    290                 295                 300

Gly Val Tyr Lys Cys Arg Gly
305                 310
```

<210> 50
<211> 6864
<212> DNA
<213> Oryza sativa

<400> 50

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cagacatgag gtgccgcccg actgacgcac atcagtcagg agtgaccggt ctgtgaccgg    180

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ggcggagtgg aggtaggtat gacccgtccc atcggagcgt gattcggagc tccctccacg    300

agaaggacgt ccaccacaag tcttcaccca tttatgaggg aatgacaggg ctgtcccccg    360

tgtcaggcag ggggcgacga tgggtcccgc ttaaggacga gcggtggctt ggcttccggg    420
```

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agtttaggaa cccttgtaac tctcaacctt aaatcccaca cacagaagta gggtattacg 660

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